PRIFYSGOL ABERYSTWYTH UNIVERSITY

The effect of mechanical perturbation on the growth and development of wheat

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Doctor of Philosophy

By

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Summary

Plants sense mechanical stimuli such as bending of the stem caused by the force of wind on the plants aerial surface. The response is known as Thigmomorphogenesis and includes a range of responses of which the most commonly reported is an immediate cessation of growth. This is followed by period of lag before growth resumes at its normal rate, which leads to shorter plants. The effect of Thigmomorphogenesis has been well characterised in diocots, and the use of mechanical treatment has been adapted for the control of height in commercial vegetable transplant production. However, the response of monocots is less understood. Wheat is the most important cereal crop in the UK, though it is prone to lodging due to factors including strong winds. Mechanical properties of the stem influence susceptibility to lodging and may be affected by exposure to repeated low-levels of mechanical perturbation. The purpose of this study was to better understand the response of monocots to mechanical stimulation. Experiments were conducted under static air greenhouse conditions and natural wind conditions outside, with brushing treatment applied using a purpose-built rig. Plants responded significantly to doses as small as 1 brushstroke. Increasing brushing dose in increments to 20 resulted in a decrease in height. Stems were significantly narrower than controls, with treatment affecting the lowest internodes most. Applying brushing during tillering stages resulted in an increase in tiller numbers, leading to an increase in the number of flowering spikes in greenhouse grown plants, but not plants grown outside. Brushing young greenhouse grown plants resulted in smaller grains in main tiller spikes. There was no effect on grain yield of plants grown outside. There was little difference in stem mechanical properties at the end of flowering, though an increase in Young's modulus was found in the stems of senesced mature plants.

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Contents

Chapter 1

1 Introduction

When animals are faced with stress they can move away and remove themselves from the stressful situation. Because plants cannot run away, they need to be able to swiftly and effectively respond to stresses in order to reduce or prevent damage. Plants respond physiologically to stress in three ways; by avoiding it, tolerating it or adapting to it. Biological processes such as photosynthesis and reproduction are very sensitive to extreme conditions, which damage internal components and disrupt chemical processes. The most well studied plant stresses include high temperature stress, drought (low-water stress), flooding/waterlogging, and nutrient limited stress. These stresses have significant economic impact on crops and commercially important plants when they affect yield or plant value. However, one stress that has received significantly less attention is mechanically induced stress. Wind, which is the main cause of mechanical stimulation, is a constant and ambient presence and shapes the form and growth of plants, while strong windspeeds causes damage.

1.1 Mechanical stress

It has long been known that the environment that plants are exposed to shapes their form, physiology and growth. These environmental conditions include water availability, nutrient

availability, temperature and light, but also includes wind. Trees on an exposed hillside may become gnarled and grow in a form shaped by the wind (Figure 1), while trees in sheltered areas grow tall, but are more likely to be blown over if the shelter is removed, or high force

Figure 1 - Trees grown on exposed hillsides become gnarled and shaped by the force of the wind

winds are applied (Biddington 1986, Jaffe and Forbes 1993). This occurs because the force of the wind overcomes the anchorage strength of the root system, resulting in the tree becoming uprooted. These observations are considered to be a consequence of the mechanical effects of wind on plants, though there are also other factors such as temperature, and water availability which may be involved (Biddington 1986).

Charles Darwin was one of the first to publish work on the responses of plants to mechanical stimuli and to identify and investigate thigmotropism, a bending or movement in response to a touch stimulus in plants. Darwin (1880) undertook many experiments using different plants to investigate their responses to touch stimuli. Using *Cassia torta* seedlings, he lightly tapped the cotyledons with a twig for a few minutes and noted that the cotyledons had increased in angle in response to the stimuli. After less than half an hour following the cessation of tapping, the cotyledons returned to their original position. Darwin also noted that small drops of water

from a syringe elicited no response from the cotyledons, but a steady stream of water did cause the leaves to move upwards. When the pot of seedlings was hit with a stick, causing them to move abruptly, the cotyledons were noted to have risen slightly. Darwin also investigated root responses to touch by placing a barrier in the path of growth. When roots came into contact (touched) the barrier, they were observed to bend away from the object. Another experiment involved exposing *C. neglecta* seedlings to a wind "sufficiently strong to keep the cotyledons vibrating" for 30 minutes. This treatment did not cause any movement of the cotyledons. Brushing the leaves of various other species caused an increased the angle of the cotyledons. However, not all species responded as much as *C. torta* and some did not show a reaction to touch at all.

Many years later, experiments by Schrank (1944, 1950) measured bending and electrical polarity in response to rubbing Oat coleoptiles. Mechanical stimulation applied to the apical 10 mm of Oat coleoptiles resulted in bending towards the stimulated side. The stimulated side became electronegative to the non-stimulated side. Jacobs (1954) observed that free-swaying trees had a greater trunk diameter and tapered more towards the base than trees that had been anchored to prevent them from swaying. These observations were supported by Neel and Harris (1971), who found that trees grown in dense stands tended to have a much narrower trunk, with less tapering and a greater height than trees growing in the open. Turgeon and Webb (1971) investigated the effect of mechanical stimulation on Squash plants. Plants were treated daily by shaking the petioles for 30 seconds and stroking the upper surface of the leaf blades with fingers. They found that both the stems and petioles of treated plants were significantly shorter in length than untreated plants. The fresh weight of petioles

and stems was also lower for treated plants than the controls. An increase in the radial growth of treated plants was also noted. Young tissue appeared to be most sensitive.

Jaffe (1973) produced the first significant paper on the topic of plant responses to mechanical stimulation (touch), where he defined the term Thigmomorphogenesis as a 'morphogenic and nastic response to touch'. In this paper, Jaffe investigated the response of 14 different species, including wheat, barley and maize, to mechanical stimulation. He subjected plants to rubbing of the stem for 11 days and measured elongation, finding that mechanically stimulated frequently had significantly reduced elongation compared with unstimulated plants. In this study, the most affected species were Bryonia, Cherokee bush bean and Marketer cucumber, where the growth of treated plants was inhibited by 70%, 45%, and 43% respectively. Neither treated Jack-o-lantern pumpkin nor Alaska pea were significantly affected by the treatment. The growth of the cereals Barley, Rye and Maize were significantly inhibited (42%, 35%, and 28% respectively), however, wheat plants were not significantly impacted by the rubbing treatment.

Thigmomorphogenesis is a syndrome of responses including changes in morphology, physical structure and chemical composition. Jaffe (1980) suggests that thigmomorphogenesis may have evolved as a response to mechanical stress, as wind stressed plants tend to be more resistant to wind-induced injury. Additionally, this may be an evolved response in order for plants to become adapted to the force of the prevailing wind and in order to minimise damage caused by excessive wind speeds (Gardiner, Berry, and Moulia 2016). Plants have a phenotypic ‘development plasticity’, which includes acclimation through Thigmomorphogenesis and a process of active recovery (Gardiner, Berry, and Moulia 2016).

In the natural environment, wind is the primary cause of thigmomorphogenesis, but rain, movement of animals and other plants, and husbandry processes (such as irrigation and moving machinery/vehicles) may also have an effect. According to Jaffe (1980) the most important causes of thigmomorphogenesis are bending by the wind and mechanical rubbing of soil particles on the subsurface parts of the plant. The most common observation of thigmomorphogenic responses is a reduction in growth, more compact growth form, an increase in radial growth, reduced number of small leaves, and thinner more flexible petioles (Mitchell and Myers 1995, Jaffe 1973, Jaffe, Biro, and Bridle 1980). This effect is common whether plants are brushed, bent, stroked, compressed, twisted shaken or exposed directly to wind. Grace and Russel (1977) also found that mechanical stress produces plants with a greater modulus of elasticity than controls and with more xylem and thicker cells than controls. Following the mechanical stimulation of the plant there is an immediate increase in the rate of growth, which lasts for 2-3 minutes. Growth then immediately stops completely for 15 to 45 minutes. The growing then resumes to about half the original rate. If the plant is not stimulated again, the original growth rate will be restored within two to three days, depending on species (Jaffe 1973, 1976, 1980). On a cellular level, thigmomorphogenesis leads to changes in cell wall composition, tissue structure and mechanical properties, which contribute towards changes in morphology of the plant (Verhertbruggen et al. 2013).

The nature and extent of responses to mechanical stimuli vary from species to species and depended on the plants stage of development during treatment. The most commonly reported effect of mechanical treatment is a halt in plant growth, resulting in smaller plants than those that did not receive treatment, and this has been reported in studies subjecting a

wide variety of species to mechanical treatment. Paul-Victor and Rowe (2010) found that brushed Arabidopsis plants were 49% shorter than those that did not receive treatment. Telewski and Pruyn, (1998) shook American Elm seedlings and found untreated plants were 46% taller than untreated. Brushing reduced the height of tomato plants by up to 25% compared with controls (Garner and Björkman 1996). Smith and Ennos, (2003) found that flexing sunflower plants reduced plant height by 22% compared with unflexed ones, whereas Goodman and Ennos (1996) found that flexing sunflower plants resulted in a 7% reduction in height. Reductions in height have also been noted in monocots including maize, where Goodman and Ennos (1996) found a 9% reduction in the height of treated plants. Zhao et al. (2018) found that elongation rates of treated rice plants was significantly lower than controls, and Wang et al. (2010) also noted a significant reduction in the height of treated perennial ryegrass plants following treatment. Steucek and Gordon (1975) treated two varieties of wheat by shaking the seedlings and found that only one variety, Blue boy, showed a significant response, where the growth of treated plants was reduced by approximately 15%. However, Crook and Ennos, (1996) found no difference in stem height between supported and unsupported wheat plants in a field.

Dicots have received a great deal of attention due to potential horticultural uses of mechanical stimulation, such as strengthening vegetable seedlings before planting in the field (Björkman 1998, Mitchell 1996) or increasing the aesthetic appearance of potted plants (Latimer 1998). This has led to the development of commercial applications of mechanical treatment in the horticultural industry (Baden and Latimer 1992, Garner, Langton, and Björkman 1997, Latimer 1991a, 1991b, Schnelle, McCraw, and Schmoll 1994).

In commercial greenhouse production of ornamental plants, vegetable transplants, and potted herbs, plants are grown at high densities to make production economically viable. However, growing at this density results in elongated plants with a variable canopy height and weak stems (Garner and Björkman 1997).

Growth regulators have been the traditional method of height control in vegetable transplant production, though many producers are seeking alternatives due to a move towards organic production or pushed by the banning of some PGRs (such as daminozide in the USA) (Latimer and Thomas 1991). The search for alternatives to PGRs has included trialling the use of controlled drought and nutrient stress to modify transplant height and growth, though these treatments have long term negative effects on growth and yield (Latimer 1991).

Mechanical conditioning has also received significant attention as an option for controlling plant growth in a commercial setting, and various methods have been trialled. Shaking treatment provides an effective and standardised application of mechanical conditioning, reducing plant height, but has limited use on a large scale (Michell 1996). Shaking combined with a rubbing treatment has also been trialled, though this was more effective than shaking alone, it also has limitations for use on a large scale in a commercial greenhouse (Latimer 1998). Conditioning by mechanical impedance using a plexiglass sheet or vinyl net placed over the top of vegetable plants has been considered (Latimer 1998). This method was found to be somewhat effective, though it was laborious and interfered with watering and other plant management practices in the greenhouse.

Brushing has become the favoured method of mechanical conditioning as it is adaptable to a variety of plants and greenhouse set-ups. Several studies have trialled the use of brush based mechanical conditioning to control the height of tomato transplants. By brushing tomato plants, Garner, Langton and Bjorkman (1997) found that treated plants were 20% shorter than controls. Schnelle, McCrow and Schnoll (1994) found a 26% reduction in height, and Baden and Latimer (1992) found a 50% reduction in plant height, while Latimer and Thomas (1991) found that treated plants had a 37% reduction in stem length. Many of the studies also reported improvements in plant appearance, such as darker green leaves (Latimer and Thomas 1991), and improved plant uniformity (Latimer 1991). Furthermore, some studies noted that mechanical conditioning improved plant resilience to handling (Latimer and Thomas 1991) and resistance to wind damage when planted out in the field (Björkman 1998, Latimer 1998).

The studies outlined above used purpose-built brushing rigs with bars or plastic piping brushed over the plants, though simply using an irrigation boom to brush the plants can provide adequate mechanical conditioning (Börnke and Rocksch 2018). Supported by the studies, brushing has become a widely used technique for height control in the commercial production of vegetable transplants (Schrader 2000, Jones Jr 2007). If mechanical stimulation also has an appreciable effect on height and growth of monocots, there could be potential commercial applications of treatment.

1.2 Mechanosensing

Mechanical forces play a critical role in the growth and development of plants such as vine tendrils winding around objects to gain support, the sculpting of trees by the force of the

wind, and root direction changes triggered by surfaces or objects within the soil (Monshausen and Gilroy 2009). In all of these examples, mechanical forces play an important role in the growth and form of the plant. Plants have evolved two broad classes of responses to mechanical stimulation. The first is a rapid response system, exemplified by the rapid closing of leaves by Mimosa pudica which utilises a highly specialised mechanosensory apparatus (Monshausen and Gilroy 2009). The second is a much slower response, which is system-wide and occurs over developmental time.

Mechanosensing is the mechanism by which plants sense and respond to mechanical stimuli at a cellular level, and below. It has until recently been poorly understood due to the complex nature of this process and because of the scale at which the process occurs. consequently, there are still many unanswered questions and much of the process still remains theoretical. Figure 2 summarises the main steps of Mechanosensing.

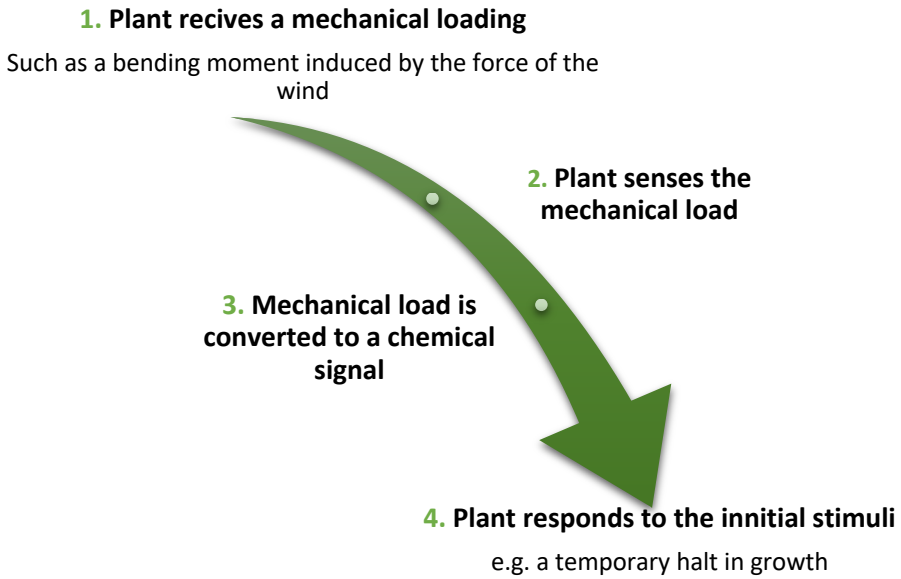


Figure 2 - Summary of the processes by which plants sense and respond to mechanical stimuli (Mechanosensing) (Onoda and Anten 2011).

Plants react almost instantly to mechanical stimuli with changes such as a halt in growth/cellular expansion, whereas, touch induced morphogenic changes occur slowly over time, and therefore may not be obvious initially, yet these changes can be profound (Coutand 2010). Braam (2005) suggested that there are two methods of mechanoperception; Firstly, stretch-activated channels might be activated by mechanical stimulation of the call membrane, which leads to an ion flux change (Jaffe, Leopold, and Staples 2002). This method is similar to mechanoperception animal and bacterial cells. Secondly, proteins that link the extracellular matrix, plasma membrane and/or cytoskeleton may act as mechanoreceptors. Alternatively, plants may use both channel activity and tethered membrane proteins to sense cellular mechanical strains, and cause ion fluxes which then act as intracellular secondary messengers. It is thought that the plant is perceiving the strain of the mechanical stress, as opposed to stress (Braam 2005). For an in-depth review of mechanosensing see (Abhinandan et al. 2018) and for a review of epigenetic responses see (Chinnusamy and Zhu 2009).

There are a number of inter- and intra-cellular signalling components, including hormones, and some potential secondary messengers, that have identified to be involved in physiological responses to touch (Braam 2005). However, the primary signal that controls the secondary messengers has not been identified at this point. It has long been known that cytosolic Ca2+ increases very rapidly after mechanical stimulation and acts as an important secondary messenger. Reactive oxygen species (ROS) have also been found to increase rapidly after mechanical stimulation and there is evidence that ROS may regulate Ca2+ channels, which suggests that Ca2+ and ROS may be 'Independently generated and functionally linked' (Braam 2005). Previous studies have implicated ethylene in the response of plants to mechanical stimulation, as it has been found to increase in concentration after mechanical stimulation

(Jaffe and Biro 1979). However, it may be a response to mechanical stimulation, but not a signalling molecule that controls the touch response (Jaffe 1980).

Touch-inducible genes were serendipitously discovered in Arabidopsis plants in the late 1980's (Braam 2005). These genes are strongly and rapidly up-regulated in expression following mechanical stimulation of the plant (Braam et al. 1997, Braam 2005). Braam et al (1990) identified 5 touch induced (TCH) genes in Arabidopsis that were rapidly induced in response to a variety of mechanical and non-mechanical stimuli. Mechanical stimuli included touching leaves, rubbing, wind, and water spray, while non-mechanical stimuli included darkness, and wounding. However, movement (translocation) of the plants and sound did not have an effect on the TCH genes. These genes have many roles (Braam 2005) including coding for Calmodulin, calmodulin-like proteins, enzymes, and other call-wall modifying proteins. TCH gene products may function in ways that affect the plants development and physiology, enabling the plant to become better adapted to its environment (Braam et al. 1997).

Three key TCH genes found in Arabidopsis are TCH1, TCH3 and TCH4, as highlighted by Braam (1997).TCH1 encodes Calmodulin (CaM), which is a major Ca2+ receptor in cells. When [Ca2+] increases in the cytoplasm, CaM binds Ca2+ and undergoes conformational changes in such a way that it is able to interact with and modify the activity of various target proteins. Therefore, CaM is likely to mediate many of the cellular changes brought about as a response to [CA2+] flux. TCH3 encodes a novel Ca2+ -binding protein, however the function and activity of TCH3 is distinct from CaM/TCH1. TCH3 May be unregulated in response to both external mechanical stimuli and mechanical stress that develops during morphogenesis. Therefore,

Braam (1997) suggests that TCH3 expression may play a role in thigmomorphogenesis. Braam further hypothesises that TCH3 may be involved in cell or tissue reinforcement or cell expansion, therefore it is likely to play a role in modifying the plant cell wall. TCH4 (Arabidopsis) encodes a cell wall-modifying enzyme. TCH4 has been identified as xyloglucan endotransglycosylase (XET) and may have an important role in determining properties of the cell wall by modifying xyloglucan polymers and incorporating them into the cell wall. Essentially, TCH4 would increase xyloglucan cross-linking with microfibrils in the cell wall, therefore reinforcing the cell wall of non-growing cells as a response to mechanical stimulation. In expanding cells, xyloglucan would be incorporated into cell walls and replace hemicellulose to maintain cell wall thickness and integrity as the cell expands.

Braam (2005) noted that there is still much to learn about the signalling pathways and transcriptional mechanisms controlling TCH gene expression. Intracellular Ca2+ fluctuation and protein phosphorylation may also play a role in TCH gene expression. Research has focused on the molecular mechanisms in dicots and specifically on Arabidopsis. Much more work is needed in order to identify if there are similar molecular mechanisms involved in the response to mechanical stimulation in monocots.

1.3 Wind as mechanical stimulation

In the natural environment, wind is the main cause of mechanical stimulation. Gardiner et al. (2016) provides an excellent summary of how wind arises in the atmosphere, produces currents and turbulences and combine with the influence of changes in topography and surface roughness to affect plants at ground level. Mean windspeed in the UK is around 8.2 knots (4.2 m/s) (Sönnichsen 2020), but varies regionally and locally due to geography,

topography and local climate. Windspeed at the Trawsgoed weather station near Aberystwyth was measured as 5.7 knots (2.9 m/s) on average between 1981 and 2010 (Met Office 2020). Windspeed at plant level is influenced by vegetation and surface conditions (Gardiner, Berry, and Moulia 2016). Vegetation covers a large proportion of land with most vegetation growing in dense communities that form canopies (deLangre 2008). These canopies may exist as homogenous groups of plants, such as a wheat-crop canopy, or a heterogeneous mix of different species. For more information on turbulence and flow in canopies see Finnigan (2000). Plant motions are a result of the combination of turbulence and drag force of the mean wind (Cleugh, Miller, and Böhm 1998). A plants wind environment changes as it grows in height and increases in surface area, therefore experiencing increased windspeeds, though this is also dependent on shelter provided by neighbouring plants. The mechanical behaviour of a plant standing alone may be quite different to a plant within a canopy due to mutual shading provided by neighbouring plants (Gardiner, Berry, and Moulia 2016).

The turbulent nature of wind in the boundary layer of a canopy results in fluctuating wind loading on plants at multiple scales, causing plants to sway/oscillate at high frequencies. If the canopy is very homogeneous, dynamic interactions arise. The paper by (Gardiner, Berry, and Moulia 2016) provides a greater depth of information on this topic. Even when plants are subjected to extreme turbulence, the resonant motion of the plant is generally not sufficient to cause the stem to break or anchorage system to fail – even though resonance may cause damage to parts of the plant. Plant susceptibility to damage is greatly influenced by stage of growth and by agronomic factors such as plant density (Cleugh, Miller, and Böhm 1998). Extent of damage is linked to wind speed, duration of wind event and regularity of high-speed

wind events as well as time of the year. Wind also causes damage such as abrasion, leaf stripping and sandblasting, but there is a limited effect on plant growth and development as plants are able to recover well.

Some plants have adapted to repeated wind loadings with the ability to streamline their form to become more aerodynamic. This decreases the drag coefficient and therefore reduces damage to the plant (Cleugh, Miller, and Böhm 1998). There are a few ways by which plants achieve streamlining. One way is that grasses such as *Pennisetum setaceum* decrease front facing surface area and porosity to become more streamline. Alternatively, shrubs like *Euonymus alatus* decrease frontal area, but increases porosity, thereby allowing more wind to flow through the canopy (Gillies, Nickling, and King 2002). Over a long period of time plants that have become streamlined to the prevailing wind may have an obvious wind-blown appearance, known as 'flagging' (Jaffe and Forbes 1993, Telewski 2012). These windswept plants have considerably less drag which therefore reduces the force of the wind and therefore reduces the potential for stem or root damage (Gardiner, Berry, and Moulia 2016). Another method by which plants are able to limit damage in high winds is by having a more flexible structure. A highly flexible structure also aids the dissipation of energy and motion through damping. Under wind loading, plants may oscillate, and this natural vibration frequency may be close to resonance with the turbulent wind's peak energy. This resonance could lead to the stem breaking or plant uprooting, therefore these oscillations need to be damped (Gardiner, Berry, and Moulia 2016).

Wind catches the leaves and exposed areas of plants, causing them to bend, flex and twist. Straining plants in this way leads to mechanical stress and long-term wind exposure can affect

plant development and alter morphology. According to Smith and Ennos (2003), wind has 2 distinct effects; Firstly It increases the amount of air flowing past leaves, secondly, it mechanically stimulates the plant by flexing the stem. In static air or where there is little air flow, photosynthesis is reduced due to a reduction in the boundary layer over the leaf which results in lower amounts of CO^2 moving over the leaf. Gentle breezes of 1 m/s or less may increase the growth rate, with an optimum wind speed of around 0.3 m/s (Wadsworth 1959). In higher wind speeds growth rates may also be reduced. When studying the effects of wind, it is often hard to separate the effects of airflow from plant movement in order to identify the specific effects of mechanical stress (Biddington 1986).

Smith and Ennos (2003) investigated the effect of air flow and stem flexure on the growth and development of sunflowers. They found that airflow and flexing had directly opposite effects on growth. Airflow increased plant height by 7% and stem conductivity by 8%, while flexing reduced height by 22% and conductivity by 16%. Where airflow reduced stem strength by 23% and rigidity by 23%, flexing increased the strength and rigidity of the stem by 26% and 12% respectively. Stem diameter appeared not to be affected by either treatment in this experiment, which suggests that there may be changes in tissue types between mechanical and hydraulic. Smith and Ennos (2003) also found that mechanical stimulation may reduce the angle of fibres relative to the long axis of the cell. This could explain the reduction on extension growth as observed in the flexed plants. They suggested that further investigations are required to look at changes in fibre and vessel structure in both young plants (as were used in the study), but also more mature plants (with secondary thickening in the cell walls).

Furthermore, photosynthesis efficiency depends upon light available for absorption by leaves and gaseous exchange through stomata on leaves. Wind has both direct and indirect impacts on photosynthesis by influencing gaseous exchange and light availability (de Langre 2008). Gentle breezes usually elevate the rate of photosynthesis slightly compared with still air or calm conditions. This is because low winds reduce boundary layer thickness, thus reducing the resistance to movement of carbon dioxide into the leaf (Smith and Ennos 2003). However, strong winds may reduce photosynthesis rates through direct and indirect methods; wind cools leaves causing them to curl up and reduce effective area, while stomata close to reduce water loss, therefore reducing the ability of carbon dioxide to enter the leaf.

1.4 Lodging

Another consequence of the force of wind on plants is lodging. Lodging is described as "the state of permanent displacement of the stems from their upright position" by Pinthus (1974),

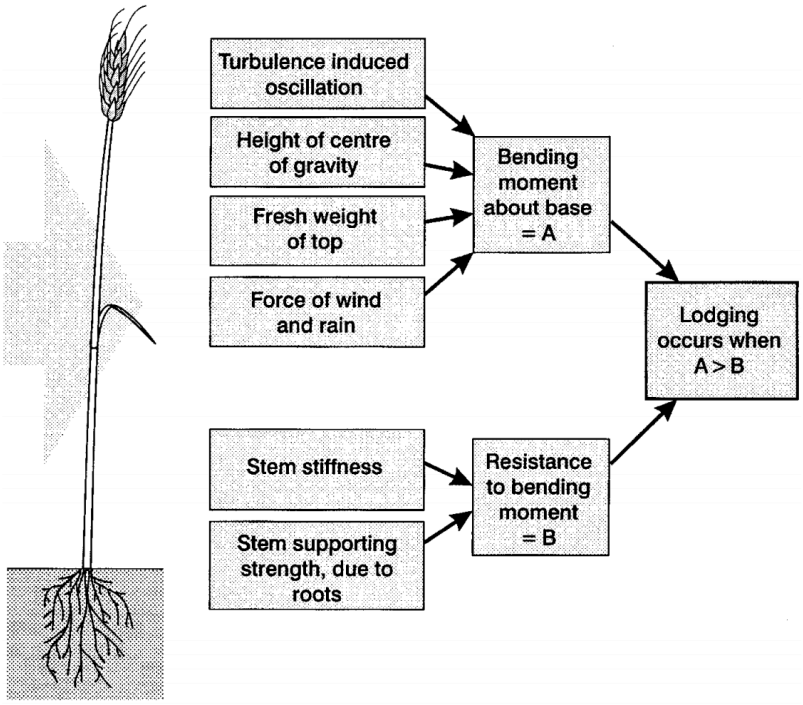


Figure 3 - from Cleugh et al. (1998) - ADAPTED from Grace J (1977) Plant response to wind. Academic Press, London, 204 pp

and can take the form of a bending or breaking of the stem at the lower culm internodes (stem lodging) or an uprooting at the plant base with a intact culm leaning from the crown (root lodging) (Sterling, Baker, and Berry 2003, Sterling et al. 2003, Pinthus 1974). Figure 3 illustrates the factors involved in lodging. It is possible for both forms of lodging to occur on the same plant or even for a stem to lodge in multiple places (Easson, White, and Pickles 1992). Stem lodging occurs when the force applied to the plant exceeds the strength of the stem. In the natural environment, wind is the primary cause of lodging, but rainfall may weaken soil round the roots, while the weight of water on seed heads increases the chance of lodging occurring. Lodging can occur at any growth stage, but the greatest losses may arise if lodging occurs during flowering or grain filling, because the plants are less able to recover. Lodging is a major problem in cereal production, reducing yields and therefore increasing the price of grain. Grain yields may be reduced by 50-80% (Berry and Spink 2012, Berry et al. 2004) and quality is significantly reduced, which is particularly a problem for bread wheat. Severe lodging episodes occur every 3-4 years, where 15-20% of planted wheat area lodges (Berry et al. 2004).

This form of damage may occur due to wind loading alone, but is usually due to interactions between wind, rain and the plant – where the weight of water on plant parts increases the chance of lodging. Lodging tends to occur towards the point of harvest, when grain heads have filled and water added to the seed head by rain or irrigation increases weight load, therefore increasing the chance of lodging (Berry et al. 2004). Lodging is also influenced by topography, underlying geology, soil type, previous crop, pests, disease, husbandry factors – such as nutrient availability and plant growth regulators (PGRs). The variety chosen may also

increase the chance of lodging, as dwarf varieties are less likely to lodge than tall varieties (Berry et al. 2004).

It is easy to see the physical damage caused by lodging when significant areas of crop lie on their side, but it is not until harvest that the true extent of damage can be measured. Due to the economic cost of lodging, this topic has received significant amounts research. The majority of research has concentrated on how and why crops lodge with more recent research focusing on how to reduce the chance of crops lodging or how to reduce yield losses if plants do lodge. Berry et al. (1998), looked at the extent and effect of lodging across several farms using aerial imagery. The study covered 6825 ha of land spread over 340 fields during the 1991-1992 growing season. They found 91% of the fields studied had lodged, resulting in 16% of the crop area lodging. The lodged area of each field varied widely from 10% to 90%. Berry et al. (1998), estimated that when this level of lodging was extended to the whole country, severe lodging could cost the UK wheat industry £60 million due to yield losses, with an additional loss of £60 million due to the reduction in milling quality of bread wheat. In comparison, Septoria tritici can result in 5-10% yield losses while fusarium infection can lead to yield losses of 5-75%. On average, fungal diseases could result in 15-20% yield losses per annum.

Lodging has a significant economic impact by reducing grain quality, increasing the cost of production, slowing the process of harvesting, increasing drying costs, and can reduce yields by up to 80% (Berry et al. 2004, Sterling, Baker, and Berry 2003, Kong et al. 2013). Lodging reduces crop yield primarily due the inability of large combines to harvest flattened plants but also because lodged plants become shaded or covered by other plants. This leads to a

reduction in photosynthesis, and increased disease susceptibility, which leads to a reduction grain size and number (Berry et al. 2004, Shah et al. 2016). Yield losses are less if crops lodge before anthesis (Fischer and Stapper 1987). Plants that lodge at this stage are generally able to re-erect their stem and grow in a more vertical direction (Berry et al. 2004). Additionally, yield losses may be greater if crops lie at a greater degree to the ground. Berry et al. (2000), found that plants that lodged less than 90 degrees had fewer yield losses than those that lodges at a greater angle.

Lodged crops become highly susceptible to fungal attack, which reduced grain quality (Shah et al. 2016) and can lead to high levels of mycotoxins in the crop, which are toxic to humans and animals (Nakajima, Yoshida, and Tomimura 2008). Losses in quality in bread wheat and other cereals such as malting barley can have significant economic costs to the farmer. Additionally, lodging increases costs by slowing harvesting and wet grain needs more drying (Berry and Spink 2012).

Stem mechanical properties influence the plants susceptibility to lodging (Berry et al. 2004). Kong and team (2013), investigated anatomical and chemical composition of solid stemmed wheat varieties alongside hollow stem varieties. Solid stemmed varieties were generally considered to be more resistant to lodging than hollow ones (Berry et al. 2004). Studies including those by Crook and Ennos (1994) and Khobra *et al* (2019) demonstrated that resistance to lodging significantly correlates with some morphological and chemical characteristics. While most of these studies have focused on solid stemmed wheat varieties, some have also looked at hollow stemmed varieties, such as by (Kong et al. 2013).

Solid stemmed varieties of wheat have thin, but hard stems where the pith is filled with undifferentiated parenchyma (Berry et al. 2004). Hollow stemmed wheat varieties have no central pith, but rather just a hollow area inside the stem. Previous research by Ford et al., (1979), found no significant anatomical differences between hollow and solid stemmed varieties and concluded that the undifferentiated parenchyma cells had thin walls and therefore would not be expected to contribute much to the structural integrity of the stem. Graham (1983) found that solid stemmed wheat varieties had smaller stem diameters and therefore a lower flexural rigidity than hollow stems. Contrary to these two studies, Kong et al. (2013), found that pith parenchyma stabilizes stems and prevents stems from becoming oval due to wind and stress pressures. Stems deformed into an oval shape are likely to buckle and collapse. Pith parenchyma may help with lodging resistance by providing some form of support to the stem, therefore preventing it from being deformed by the force of wind.

Kong et al. (2013), found that solid wheat stems were more resistant to lodging than hollow stems and there was a strong relationship between width of mechanical tissue and lodging resistance. The study also found that wide and solid stems with greater proportions of mechanical tissue were more resistant to lodging, indicating that mechanical tissue plays important role in lodging resistance in wheat. They also found that solid stemmed varieties had a greater lignin content and that there was a significant correlation between lignin and cellulose content and lodging. A previous study by Hondroyianni et al. (2000), also found a correlation between degree of lodging resistance and lignin/cellulose content. Kong et al. (2013), suggests that lignin and cellulose must therefore play an important role in lodging resistance. Berry (2007) has developed a Lodging proof ideotype based on ideal plant, stem, and mechanical characteristics for a lodging-resistant wheat plant to aid the development of

lodging resistant varieties. Furthermore, exposure of plants to mechanical treatment, such as repeated wind loadings inducing bending, may affect stem and mechanical properties related to lodging (Gardiner, Berry, and Moulia 2016).

1.5 Biomimicry and other reasons for studying Thigmomorphogenesis

Understanding how plants resist and adapt to wind and bending forces helps in the design of bioinspired products and structures. For example, an analysis of the structure and mechanical properties of wheat stems had led to the development of new beams and columns (Değer et al. 2010). Biomimicry is a meeting of biology and engineering with the aim of solving technical problems using ideas inspired by natural materials and processes. Martone et al. (2010), provides a useful summary on biomimicry and some biological solutions for engineering problems. Adaption through evolution to survive constantly changing environments has led to the development of organisms, structures and features which have become optimised for a particular, environment, role or function. Using plants as inspiration, engineers and designers can create novel design solutions based on evolutionary plant mechanical design. Such solutions include deformable and foldable structures, self-repairing membranes, and smart materials (Moulia 2013). Biomimicry principles could also be applied to building structure design, where ideas from nature can be used to construct buildings that are able to withstand high force winds, such as hurricanes and vigorous shaking from seismic activity. The Taipei 101 building is an excellent example of biomimicry in building design. The tower has been constructed with reference to stems of bamboo and contains a heavy pendulum in the centre of the building to dampen oscillations created by wind and earthquakes.

1.6 Wheat and why it is a good candidate for study

The three most important food crops in the world are maize, rice, and wheat, and together they make up 90% of the worlds grain production (Wrigley 2010). Wheat was the first cereal crop to be domesticated and the storability of these grains may have been a major catalyst in the transition of humans from hunter-gatherers to settled agricultural communities. Since its domestication in the fertile crescent over then thousand years ago, it has become a staple carbohydrate and protein source for billions of people around the world. Global wheat production in 2017 covered 218 mega hectares of land, producing a total of 771,718,579 tonnes at an average of 3.48 tonnes per hectare. In the same year, 1.8 mega hectares of wheat were cultivated in the UK, at an average yield of 8.15 tonnes per hectare (FAOSTAT 2019).

Wheat is the most important cereal in the UK and as a highly versatile crop its grains can be milled into flower and processed into a wide variety of products – from bread and pasta to cakes and biscuits. A vast range of varieties of wheat have been produced to fulfil these varied end-uses, with protein content being an important factor for determining grain use (Batey 2017). Additionally, the whole plant can be utilised – for animal feed and bedding, construction materiel and as a fuel (Uthayakumaran and Wrigley 2010). Waste materiel can also be converted into liquid fuels and platform chemicals.

The focus of research for wheat has been on increasing grain yield and quality as well as the biotic and abiotic stresses that affect these traits. Environmental stresses such as drought and high temperature affect plant growth, reduce yield and affect grain quality. Wheat is also susceptible to lodging, another factor that reduces grain yield and quality. Climate change

means there is a need to develop and adopt more stress-tolerant varieties (Gooding 2010). Furthermore, wheat research and breeding needs to focus on improving yield and stabilising grain quality while reducing inputs, such as nitrogen fertilizers, and improving opportunities for biofuel production (Shewry 2009).

As outlined in previous sections, wind is a significant environmental factor that induces bending moments in plants and leads to mechanical stimulation. There are still some gaps in our understanding of how mechanical stimulation affects the growth and development of wheat. Further, recent developments in techniques such as μCT scanning can now give unique insights into traits such as individual grain shape and size which may be affected by mechanically induced stress.

Within the topics of wheat and mechanical stress there remain many questions that still need to be addressed, such as; How does wheat respond to mechanical stimuli on a phenotypic, structural, tissue or cellular level? Is there a minimum threshold for the perception of mechanical stimuli? Does wheat become desensitised to repeated loadings? Does the effect of manual brushing differ to natural wind stimulation? Does plant age affect the response? Also due to the key reasons for studying wheat as outlined above it's also important to consider the effect of mechanical stimulation on grain yield, stem mechanical properties and lodging. The benefit of conducting this research would be to further understand how monocots respond to mechanical treatment, and to examine the effect of mechanical treatment on lodging related properties, such as stem mechanics and stem length. As mechanical conditioning is used in transplant production to strengthen seedlings, could mechanical treatment applied to wheat plants affect lodging related properties and reduce

the incidence of lodging. Furthermore, if mechanical conditioning is significantly effective in controlling plant height, it could be applied on field scale and reduce the need for growth regulators.

The aim of this study is to observe the effect of mechanical stimulation on the growth and development of wheat plants, and consequences of mechanical treatment on stem mechanical properties and grain yield.

Chapter 2

2 Shared methodologies

This chapter details methodologies that were used across multiple experiments. More specific methodologies, including greater detail of measurements taken and data collected, is included in each experimental chapter.

2.1 Variety choice

For preliminary experiments, the winter wheat variety JB Diego (Senova seeds) was selected from the AHDB recommended list (AHDB 2016). This variety was popular among farmers (Impey 2012) and used as a control variety for comparing other varieties of winter wheat performance against. For the later experiments (chapters 4, 5, and 6), the spring variety Mulika (Senova seeds) was selected from the recommended list. As this variety does not require vernalisation, the effect of treatment on growth, flowering and yield could be studied in a shorter time than for winter wheat. A popular spring bread wheat amongst farmers, Mulika is also frequently used as a control variety in trials. A field stand of both varieties is shown in Figure 4.

Figure 4 – Stands of JB Diego (left) and Mulika (right) wheat at Cereals Event 2017

2.2 Plant preparation and growing conditions

Seeds of JB Diego were sown into 3 inch plastic pots containing John Innes Number 3 compost. Seeds of the Mulika variety were sown into 5 inch plastic pots contacting the before mentioned compost. Each pot contained one seed, with any non-germinated plants discarded 7 days after sowing. Plants were placed in watertight trays, where water was added to keep the plants hydrated. Trays were topped-up daily. Watering the plants in this way prevented disturbance of aerial plant parts therefore keeping plant movement to a minimum.

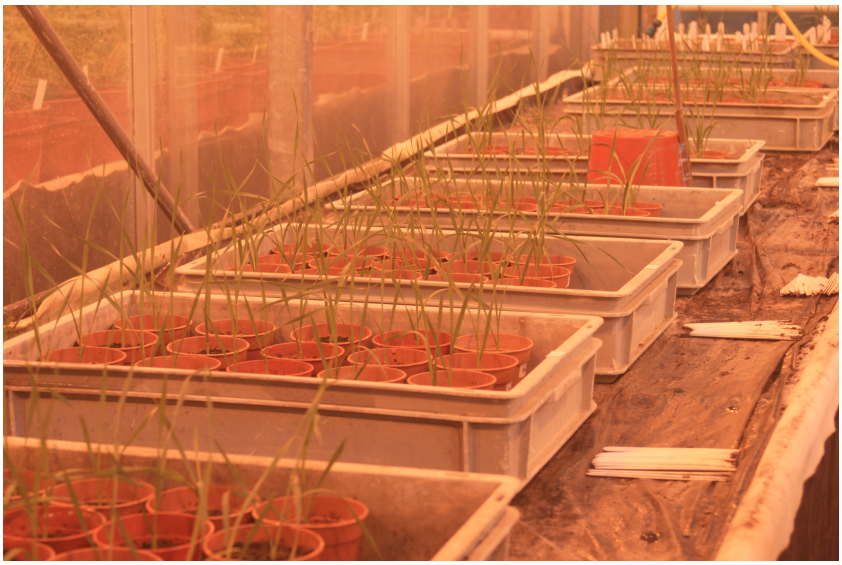


Figure 5 - Seedlings of JB Diego, 2 weeks after seeds were sown and prior to assignment to groups and treatments.

Where plants were grown in a greenhouse (Figure 5), either for the entire duration of growth, or just for the emergence and establishment phases of growth, additional lighting was provided (10-hour photoperiod) and supplementary heating (25°C day/ 10°C night). The aim of supplementary lighting was to maintain a reasonably consistent light level and temperature level within the greenhouse throughout the duration of the experiment.

Once plants reached the 3-tiller stage, the main tiller (first to emerge) was tagged with a brightly coloured piece of string (Figure 6). This aided identification of the main tiller for consistency of measurements throughout the experiment.

Figure 6 – (A) Wheat plant at 3 tiller stage, when main tillers were marked using a piece of brightly coloured thread. (B) Tagged main tillers were easily identified at later growth stages.

2.3 Application of treatments

2.3.1 Simulated wind

Simulated wind was produced using an Addvent 18-inch domestic floor standing fan and measured using an Omega HHF11A handheld anemometer (Figure 7). Plants were positioned at least 25 cm from the fan. Rows were rotated forwards (front row moved to the back, therefore bringing the second row to the front) each day, so that all plants received equal as possible wind treatment, including those further back. Wind speed at canopy height in the centre of the group of plants measured an average of 3.5 m/s.

Figure 7 -Simulated wind was produced using a floor standing domestic fan. The airflow measured 3.5 m/s at canopy level in the centre of the group of plants.

2.3.2 Brushing

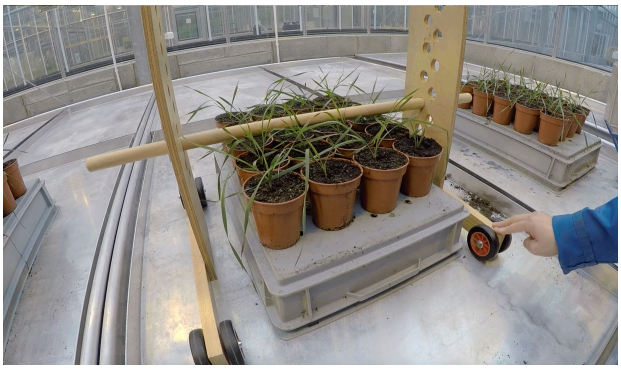
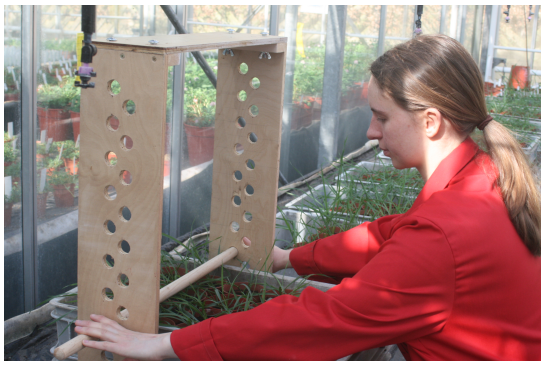



Figure 8 - Brushing applied using a purpose built manually controlled brushing rig. The bar flexed the plants at approximately half canopy height.

Brushing treatment was applied using a purpose-built rig designed to be manually controlled and pushed/pulled forwards and backwards to bend and flex the plants (Figure 8). The design was based on similar plant flexing rigs used by Baden and Latimer (1992) and Latimer and Thomas (1991). Each 'brushstroke' consisted of one forward pass and one return pass of the beam over the top of the group of plants. 20 brushstrokes were applied during a period of 1 minute and 30 seconds. The bar was raised as plants grew to ensure plants were brushed at approximately half the canopy height and to prevent damage to the base of the plants.

2.3.3 Static

When not receiving treatments, plants were placed on a bench in the greenhouse under 'static' conditions (Figure 9). Ambient windspeed within the greenhouse was measured as less than 0.3 m/s. Control plants received no treatment but were grown alongside those that did.

Figure 9 - Plants were arranged into groups and placed in trays, which were randomly placed along the bench. When not being treated, all plants grew in 'static' conditions.

2.4 Phenotypic measurements

2.4.1 Growth stages

Stages of growth were determined as according to the Zadok's scale and the AHDB Wheat growth guide (2018).

2.4.2 Height

Canopy height

Prior to stem extension, the plants consisted of leaves and tillers. At this point, canopy height was measured in mm using a meter ruler as the distance between the upper boundary of the main photosynthetic tissues on a plant and the soil surface (Pérez-Harguindeguy et al. 2013). When height was measured during stem extension, but prior to flower emergence, height was measured in the same way, but included emerging inflorescences.

Plant height

Once the spikes had emerged, plant height was measured in mm using a meter ruler as the distance between the upper boundary of the main photosynthetic tissues on a plant and the soil surface excluded the inflorescences.

2.4.3 Tiller count

Tillers are side shoots that emerge at leaf-stem junctions (Figure 10), with the first tiller emerging from the junction of the first leaf when the plant is at the 3 leaf stage (Bell and Fischer 1994). The next tiller emerges from the second leaf and each further tiller emerges from within the leaf sheath of each subsequent leaf. Secondary tillers develop from leaf junctions in primary tillers (AHDB 2018).

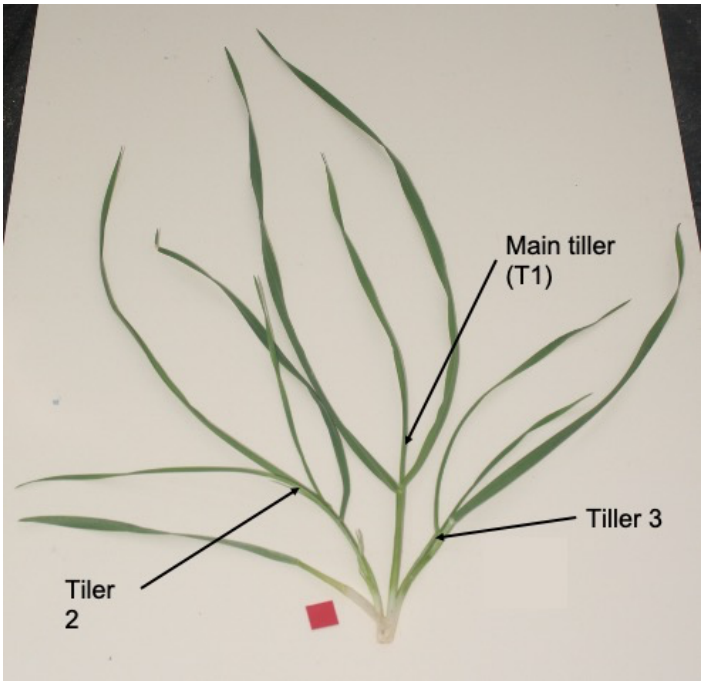


Figure 10 – Tillers emerge at leaf junctions. An example of tiller numbering at the three-tiller stage.

Stress affects the pattern and rate of tiller production and number of tillers produced, particularly when stress is applied during early growth stages. Counting the number of tillers produced gives an idea of how plants respond to stress and allocate resources during early stages of growth.

Environmental conditions and stress affect final tiller number. Once stem elongation begins (GS30), tillers begin to die off, starting with the most recently produced. Tillers produced towards the end of the tillering phase are rarely fertile.

2.4.4 Spike count

The number of flowering spikes produced by a plant is related to the number of tillers produced. More tillers generally result in more spikes, but stresses during tillering and later

growth stages can result in tiller death and a subsequent reduction in the number of flowering spikes. Fully emerged spikes on each plant were counted, however there were some spikes that failed to fully emerge or died during flowering and these were therefore excluded from the count.

2.4.5 Leaf measurements - Leaf length, width and area

Leaf length was measured along the centre of each leaf, from the ligule to the tip using a 300 mm ruler. Maximum leaf width was measured at the widest section of the leaf using a ruler accurate to 1mm. For an estimation of leaf area, the following equation was used,

(Equation 1):

$$a = l \times w \times k$$

L, length and w, width, where k is a constant, 0.75 (Bell and Fischer 1994, Chanda and Singh 2002).

The flag leaf is the last leaf to emerge and the last to senesce and coupled with its position at the top of the plant provides the greatest amount of light interception of all leaves on the stem (Gooding et al. 2000). Flag leaf area (Figure 11) is therefore important for photosynthesis and carbohydrate production for the growing spike. Measuring leaf length gives an indication of the impact of stresses on photosynthetic organs. Reductions in flag leaf length and especially area, impacts on the photosynthetic capacity of the plant, and ultimately affects carbohydrate accumulation and grain yield.

Figure 11 – Main tiller flag leaf length (l) and width (w) were measured using a 300mm ruler to the nearest mm.

2.4.6 Internode measurements

Internodes were numbered from bottom to top. The greatest number of internodes found on a stem of Mulika was 4, therefore the top internode of all plants was numbered '4', as shown in Figure 12. The length of each internode was measured in mm as the distance between its bottom and top node using a 300mm ruler. The internode diameter was measured at 6 locations along the internode using callipers accurate to 0.001mm. The 6 diameter measurements were averaged to give a single value for each internode.

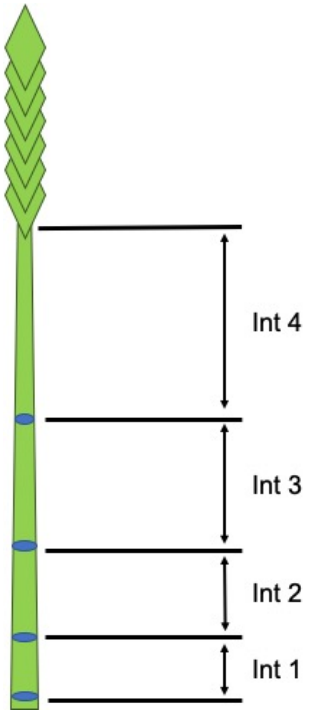


Figure 12 - Internodes (Int) were measured from bottom (1) to top (4) as tillers of the wheat variety Mulika consisted of a maximum of 4 internodes.

2.4.7 Biomass measurements

Plants were cut at their base (at or just slightly under the soil surface) and separated into above and below ground material.

2.4.7.1 Above ground biomass

Fresh above ground material was weighed (fresh weight), then dried by placing the samples in an oven at 60°C for 48 hours and weighed again (dry weight). The moisture content was calculated on a fresh weight basis using the following equation (Equation 2):

moisture content % = [(fresh weight (g) – dry weight (g)) / fresh weight (g)] * 100%.

2.4.7.2 Below ground (root) biomass

For below ground biomass measurements, 5 plants from each treatment were randomly selected. The roots from the selected plants were separated from the soil by firstly gently breaking up the soil mass, then soaking the soil/root mass in water, to loosen soil from the roots. The remaining soil/root mass was placed on a fine sieve, under a running tap, and gently agitated to loosen and remove the remaining soil. Once the roots had been cleaned, excess water was removed by placing them on a paper towel, and samples were dried in an oven at 60°C for 48 hours and dry weight was determined.

2.4.8 Spike measurements – number, length and weight

Spike length was measured at both end of flowering (T2) and at maturity (T3). The length of spikes was measured in mm from the base of the rachis to the top of the terminal spikelet, excluding any awns.

2.4.8.1 Spike weight

Spike weight was examined only at maturity. The spikes on each plant were labelled and cut from the stem. The main tiller spike from each plant was then weighed individually, and together with all other spikes from the same plant to determine total spike weight.

2.5 Plant biomechanics

2.5.1 Stem mechanical properties background

The Young's modulus, also referred to as the modulus of elasticity, is a material property that describes how stiff a material is. The Young's modulus is calculated by measuring the stress and strain of a material when a force is applied and dividing stress by strain. To obtain these measurements, a test piece of the material is prepared of known length and cross-section area and placed into a test rig that applies a force to the material. Stress is calculated as the load recorded at each stage as it is applied to the material, divided by its cross-section area. An extensometer, clamped to each end of the test-piece, measures the length of the material before, and after application of the load. Strain in the material is calculated as the increase in length of the material after loading, divided by its original length.

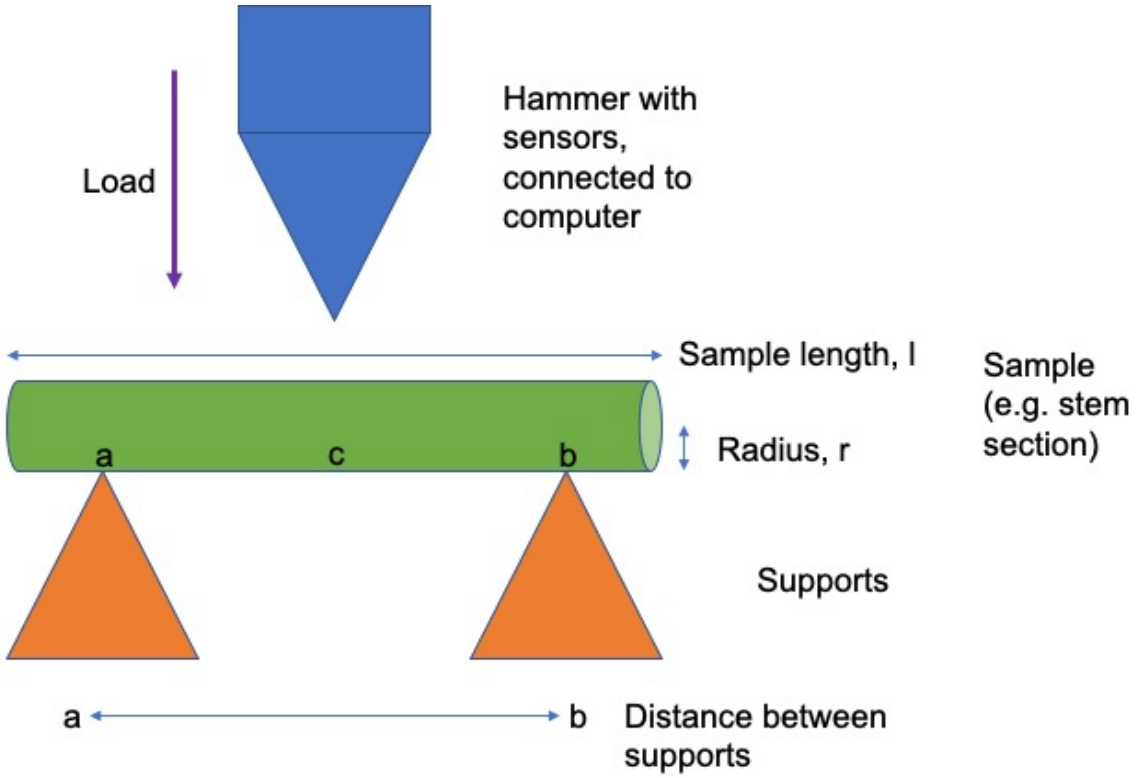


Figure 13 - Diagram of a 3-point bending set-up. Sample of length l, and radius r, placed on supports a and b with a load lowered towards the centre of the sample, between the two supports (c).

Young's modulus may also be estimated in a similar manner as described above, but without the use of an extensometer. A 3-point bending test may be used to obtain measurements of the mechanical properties of the stem (Figure 13). 3-point bending tests involve a test-piece with known length and cross-section area suspended across two points, subjected to a load of known size placed centrally between the supports, while the force applied at each point and distance that the sample is deflected are measured.

Data from the 3-point bending tests is also used to calculate both bending stiffness and bending rigidity of the sample material. Bending strength is defined as a materials ability to resist deformation under load and is based on the maximum force before failure. Samples with a greater bending strength are stronger and more able to resist deformation under a

load. In the 3-point bending method used in this study, failure was defined as the point on the force/deflection graph at which load began to decrease with increasing deflection. At this point, the stem had received irreparable damage to its structural integrity (e.g. crushed or buckled cell walls) (Robertson et al. 2014).

Bending rigidity (EI), also known as flexural rigidity, is the inability of a material to be bent or forced out of shape and is based on the moment required to bend a structure one unit of curvature. Essentially, it is the resistance that the structure offers when undergoing bending. It is a function of the Young's modulus (E) and the second moment of area (I).

2.5.2 Stem mechanical properties methodology

Main tillers collected at both the end of flowering and at the senesced stage were immediately used for mechanical testing, to avoid wilting, which could affect the mechanical properties of the samples. All leaves and the leaf sheath were stripped from the tiller to leave just the stem. For internodes 90 mm or longer, an 80 mm section was cut from the centre of the second and third internode of each main tiller. For shorter internodes, a 50 mm section was removed (Figure 14). The sections were then subjected to a three-point bending test using a mechanical texture analyser (TA.XT plus, Stable Micro Systems) equipped with a 50N loading cell, as shown in Figure 15. Stem section samples were placed across two supports, with the metal test probe hammer suspended midway between the supports, and 30 mm above. Where stem sections were measured at 80 mm in length, the supports were 50 mm apart, and 50 mm long sections rested on supports 30 mm apart. The test probe was lowered

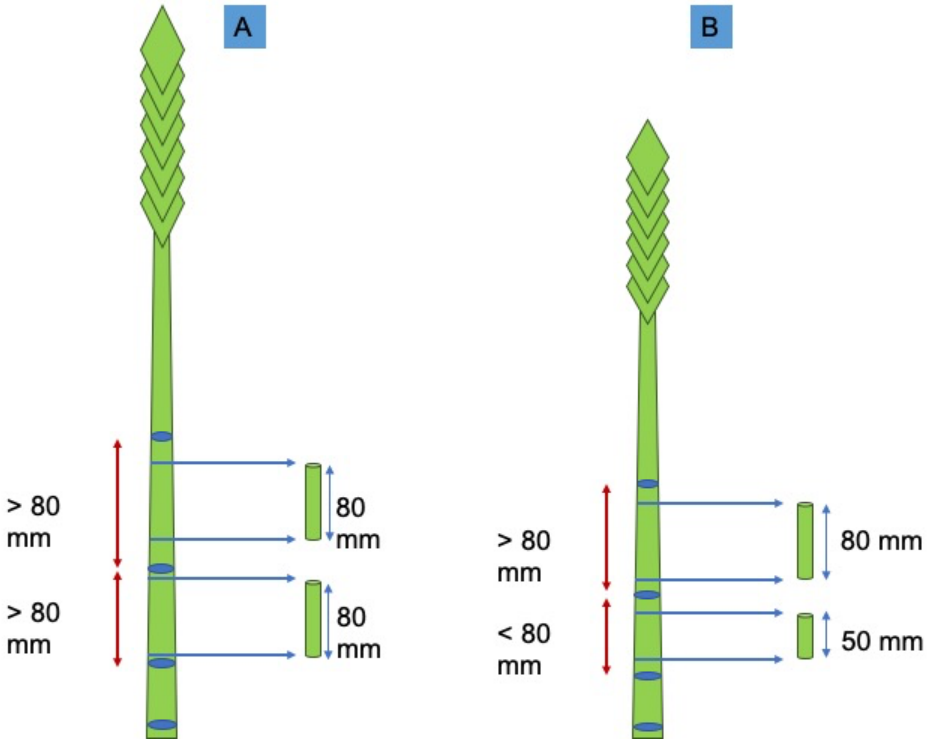


Figure 14 – Sections were taken from internodes 2 and 3. Where the internode was more than 80 mm in length, a section of 80 mm was taken, but if the internode was shorter than 80 mm, a section of 50 mm would be taken.

towards the centre point of the sample with an initial speed of 49.8 mm/min. Once the probe touched the sample, speed was slowed to 15 mm/min to slowly bend the sample. The initial probe drop speed was greater than test speed to reduce the time taken to process each sample. Exponent-TEE32 software produced a graph detailing force applied to the material (N), and deflection (mm) of the material. This data was used to calculate the modulus of elasticity (Young's modulus), bending strength and bending rigidity of the material, as detailed in section 2.5.3. After 3-point bending, the fresh sample sections harvested at the end of flowering were then placed in 70% ethanol, while senesced samples were stored in a container until cross section measurements could be acquired.

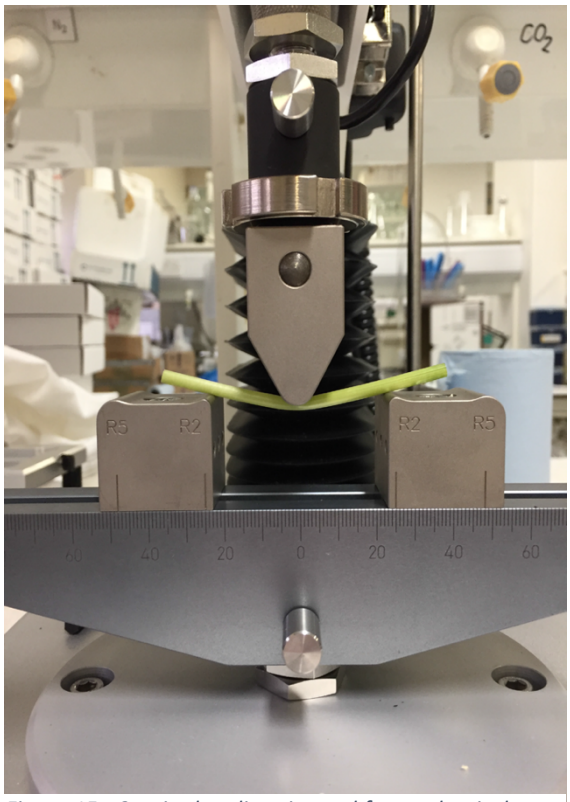


Figure 15 - 3-point bending rig used for mechanical tests. 80 mm long stem samples rested across two supports 50 mm apart. The test probe was lowered onto the centre of the sample and continued its descent at 15 mm/min for 6 mm, bending the sample, and producing a force/deflection graph on the attached computer throughout its fall.

Thin stem cross sections were taken with a sharp razor blade from close to the site of impact. Sections were mounted dry onto clear glass microscope slides and viewed with a 10x objective under a Leica MZ6 microscope. Images of the sections were taken using an attached Nikon Coolpix 990 camera in JPG format (Figure 16). In addition, images of a calibration scale (1 cm) were taken in order to calibrate software measurements later. Images were then analysed using ImageJ (Schneider, Rasband, and Eliceiri 2012) and calibrated using the calibration images to calculate external diameter, external radius, internal diameter, internal radius and stem thickness (internal boundary to external boundary transect) where samples were hollow. Pith filled samples were measured for external diameter and radius.

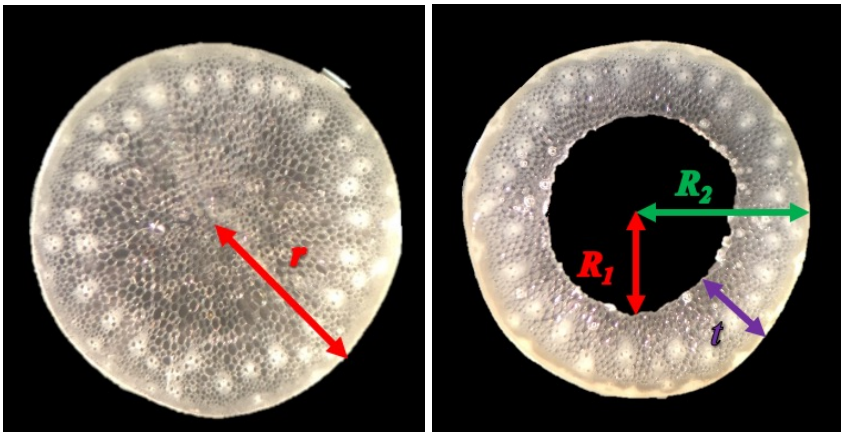


Figure 16 – The radius (r) of stem sections containing a pith was measured (left), while both the internal (R1) and external radius (R2) of hollow stem sections was measured (right). Stem thickness (t) for hollow sections was calculated as R2-R1 and therefore was a measure of the internal stem boundary to the external stem boundary.

Data from the three-point bending and cross-section measurements were then used to calculate mechanical properties of the stem including second moment of area, bending rigidity, bending stress, bending moment, and Young's modulus.

2.5.3 Mechanical calculations

Using internal and external radius measurements, the second moment of area, I, was calculated (Equation 3):

$$I = \frac{1}{4\pi}(R_2^4 - R_1^4)$$

Where R_2 was the external radius and R_1 the internal radius of the stem sample. For non-hollow, pith filled samples, only the radius, r, was needed to calculate I (Equation 4):

$$I = \frac{\pi}{4}r^4$$

The Young's modulus was then calculated using data from 3-point bending; force applied, F, distance between the supports, L, and deflection of the sample at centre, δ, using Equation 5:

$$E = \frac{FL^3}{48\delta I}$$

Next, the bending strength, S, was calculated (Equation 6):

$$S = \frac{F_{max}L}{4}$$

where F_{max} was the maximum force the sample withstood before failure and L, the distance between supports.

Finally, the bending rigidity, EI, of the stem section was measured (Equation 7):

$$EI = \frac{L^3\left(\frac{dF}{dY}\right)}{48}$$

Where dF/dY was the slope of the force-deflection curve.

The equations used here are based on those presented by Gordon (2009) and Crook and Ennos (1996) and have been adapted to take into account the geometry of hollow stems.

Additionally, cross-section area of the internode section was calculated:

Area (A) of hollow stemmed sections (Equation 8):

$$A = \pi(R_1^2 - R_2^2)$$

Area of pith containing stem sections (Equation 9):

$$A = \pi r^2$$

Stem thickness was a measure of the radius of the cross-section of sections containing a pith and as outer radius minus inner radius (r2-r1) for hollow sections.

2.5.4 CT scanning and grain analysis

Main tiller spikes harvested from the mature plants were analysed using micro CT scanning. Some spikes were cut into two equal sections in order to fit into the CT scanner. Data from each half was then combined to reconstruct the whole spike.

Originally designed for medical applications, μCT (x-ray micro computed tomography) is an imaging technique based on differential x-ray penetration of materials of differing compositions and densities. It is a non-invasive and non-destructive method which can yield detailed 3-dimensional images of internal structures. μCT scanning can be a useful tool in the study of complex plant morphology, especially as the process can be partially or fully automated (Hughes et al. 2019).

Very little is currently understood about phenotypic variation in wheat grain shape (Hughes et al. 2019), and this is probably due to the difficulty studying these traits using manual measuring or traditional imaging techniques. Individual grain dimensions are tricky to measure by hand due to the size of the grain, and even utilising image analysis can be hugely time-consuming. μCT scanning is a fast and accurate imaging technique and combined with an image-analysis programme it can be used for detailed study of grain characteristics in-situ in the spike. This method utilises the entire spike and may be used to study grain position and distribution as well as grain morphology within the spike.

Grain size and morphology are becoming increasingly important agronomic traits along with grain number per spike, which has a major impact on overall yield. Spike analysis using μCT

scanning speeds up trait measurements and allows for a greater variety of grain traits to be measured that would be tricky or impossible to measure otherwise (such as circularity and crease depth).

μCT scanning has been successfully utilised for imaging cereal spikes, and in combination with an image analysis pipeline, data on spike and grain characteristics can be extracted and analysed (Hughes et al. 2017, Strange et al. 2015). However, research on grain morphology utilising this technique is generally limited to proof of concept and testing the down-stream image processing method. Most papers have therefore focused on testing and evaluating the method rather than applying it to analysing novel experimental or treated plant material.

This technique was evaluated in a pilot study by Strange et al (2015) to identify grain morphological differences between several varieties of wheat. Hughes et al (2017) also tested the method by subjecting wheat plants to high temperature and two watering regimes and analysed the effect of the treatments on grain morphology. Hughes et al (2019) used the technique to examine differences in grain morphology between domesticated and wild varieties of wheat and barley. These studies have found the technique to have a high degree of accuracy (95-99% accurate (Strange et al. 2015)), when compared against manual grain counts (Hughes et al. 2017). While these studies have evaluated this technique and found it to be a very effective tool it remains underutilised in the study of wheat grain morphology.

Other applications of the μCT scanning method include that described by Le et al (2019) who used this technique to observe grain development and morphological changes in wheat grain

at different stages of development. μCT scanning has also been applied to study stem traits in sorghum and Miscanthus (Gomez et al. 2018, da Costa et al. 2019).

In this study, μCT has been used to analyse grain characteristics including length, width, volume, and grain count to determine if there are differences between mechanically stimulated and untreated plants.

Main tiller spikes from each of the harvested plants were selected for μCT scanning. Some spikes were too long for the holder and therefore were cut in half and each scanned separately. Samples were loaded into individual holders measuring 34x70 mm and scanned in batches of 12 using a μCT1000 scanner (Scanco medical, Switzerland), for detailed information on the conditions used, see Hughes et al., 2017. Output images were produced in a propriety ISQ format (Scanco medical, Switzerland). MATLAB-based software developed by Hughes et al, was used to perform feature extraction and is available from github https://github.com/NPPC-UK/microCT_grain_analyser. Features extracted included individual grain length, width, depth, volume and surface area. Additional information yielded by the software included 3-dimensional position of each grain in the spike using x, y, z axis position.

Isolated grains identified by image analysis were orientated by calculating the major axis of the 3D shape, which was a measurement of connected pixels and labelled grain length. Grain width and depth involved examining a cross-section of the grain and measurements of the major and minor 2D axis (Figure 17). Grain volume was a measurement of complete

connected-pixel count for each grain. Grain parameters in pixels were then converted to mm using the formula (Hughes et al. 2017) (Equation 10):

$$mm = \frac{(pixel \times coversion)}{1000}$$

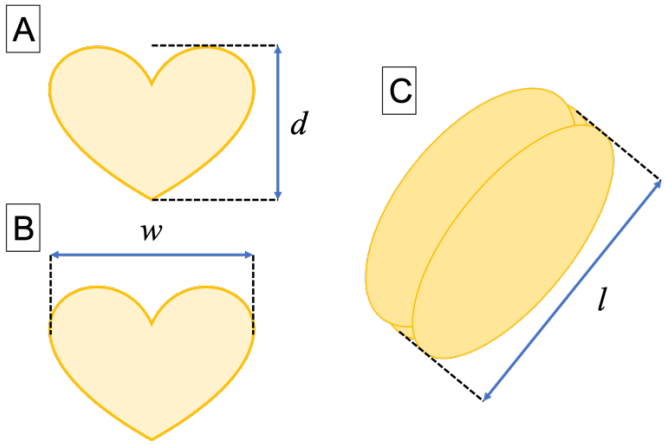


Figure 17 - Individual grain measurements. Images A and B showing depth and width measurements of the minor and major axis of the 2D image. Image C shows length measurement obtained by calculating the major axis of the 3D shape.

Data was cleaned up to remove false positives by eliminating identified outliers using the top 0.025 upper and lower percentiles. Data for each half of separated spikes was recombined to give an output for an entire spike. Grain count was inferred from the number of volume measurements for each spike.

After scanning, a random 10% of the spikes imaged were selected and the number of grains in each of these spikes was manually counted. This grain count was then cross-checked against the number of grains for each of these spikes picked up by the software. The number of grains found by the software was found to be 95% accurate against those manually counted.

2.5.5 Statistics

All analysis was performed using SPSS statistics (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Treatment effect was analysed using analysis of variance (ANOVA) at the 5% level ($p<0.05$) of significance. Where ANOVA indicated a statistically significant difference, post hoc tests using Tukey's HSD and Dunnett T were conducted. Tukey tests conducted pairwise tests between all treatments, including control, while Dunnett T tests compared each treatment against the control/untreated plant data.

In Chapter 5, where treatment effect on plants of different ages was compared, T-tests were used to compare treated and untreated plants of each age group at the end of treatment.

Chapter 3

3 Preliminary experiment – Comparing wind and brushing

3.1 Introduction

In the natural environment, wind is the main cause of mechanical stimulation. Wind causes dynamic loading, forcing upright self-supporting plants to bend and flex, thus exerting mechanical stresses on both individual plant parts and on the plant as a whole. Plants have adapted to survive in windy conditions by sensing wind loading and responding effectively with changes to growth or plant structure, in-order to reduce damage. These responses include thickening stem growth, reducing vertical growth, re-orientation and reconfiguration of the canopy to become more streamline. Even once damaged, plants including cereals are easily able to recover (Gardiner, Berry, and Moulia 2016). Additionally, wind affects the plants microclimate, affecting temperature and moisture within the plants canopy. Furthermore, wind increases airflow across the plant (Smith and Ennos 2003) and this in turn affects the leaf transpiration rate (Retuerto and Woodward 1992). Grace (1974) found that increasing windspeed from 1 m/s to 3.5 m/s resulted in an increase in night transpiration rate of up to 10 times in Festuca arundinacea plants. By far the greatest and most obvious effect of wind

is the damage that it can cause. This can range from serious whole-plant damage (bent, buckled or broken stem or trunk) to the loss of branches or limbs, from which the plant may be more able to recover. Damage to the plant may also occur where plant parts collide causing folding and taring of the leaves, and breaking of stems and petioles, though plants may easily recover from these outcomes.

Under controlled conditions, a wind-like airflow can easily be reproduced by using a fan (domestic, commercial or in wind-tunnel form) to mechanically stimulate plants. Previous experiments have used wind tunnels to induce motion in either individual plants or groups of plants (Grace and Russell 1982, Retuerto and Woodward 1992). However, wind tunnels require large amounts of space in order to focus airflow in a small area, which limits their use where space may be an important consideration (like in a greenhouse for example). Alternatively, various forms of domestic type fans have been used, with plants simply placed in front of the fan for treatment (Anten et al. 2010, Smith and Ennos 2003, Pigliucci 2002, Venning 1949, Henry and Thomas 2002). However, wind produced by fans may produce an increase in airflow, but it does not simulate the effect of natural wind exactly. Wind is dynamic in nature and fluctuates in speed and continuity. Fans produce a comparatively consistent and continuous wind speed and flow, therefore wind produced by these fans will be discussed as 'simulated wind' in this chapter. Mechanical stimulation can also be applied using a variety of alternative methods, including bending (Gartner 1994), brushing with solid sticks and beams (Schnelle, McCraw, and Schmoll 1994, Latimer and Thomas 1991), soft dusters (Anten et al. 2010, Liu et al. 2006), rubbing (Jaffe 1976) and shaking (Mitchell 1996). Treatment can be applied to individual plant parts such as leaves or petioles (Peacock and Berg 1994), to large areas, such as stems (Huber et al. 2014), or to the plant as a whole (Baden and Latimer

1992). Brushing induces a bending moment in the plant and supplies mechanical treatment without significant impacts on the microclimate (Anten et al. 2010).

Bending plants using a purpose-built rig is a highly effective method for the treatment of a wide variety of plants, as demonstrated by Baden and Latimer (1992) Garner and Bjorkman, (1996), Paul-victor and Rowe, (2010) Morel et al., (2012), and Börnke and Rocksch (2018). This method of brushing provides a measurable, consistent, and repeatable application of mechanical perturbation but also provides an opportunity for automation, which would reduce the chance of human error in treatment and improve consistency (Paul-Victor and Rowe 2010, Morel et al. 2012). Treating plants by brushing areal parts has the potential to cause damage. However, this can be limited by use of non-adhesive surfaces for brushing instruments and by adjusting the height at which plants are treated to prevent overburdened stems from snapping (Schnelle, McCraw, and Schmoll 1994).

Wind has two distinct and quite different effects on plants - it induces movement and increases airflow over the leaves. Many of the papers already mentioned studied the isolated effect of bending and flexing in the absence of wind, while fewer have analysed the effect of airflow with the absence of flexing and bending. This is likely due to the difficulty in treating plants with an increase in airflow, while ensuring that the plants do not bend or move. Equally, it is not possible to study the direct mechanical effect of wind without having an increase in airflow, unless the mechanical treatment is replicated using an alternative method of treatment such as brushing.

Some studies have attempted to evaluate and compare wind with its duel aspects of airflow and mechanical treatment, with purely mechanical treatment in the absence of an increase in airflow. One such study was by Smith and Ennos (2003), where the effects of airflow and stem flexure were investigated in conjunction. They observed the effects of wind produced by a domestic fan, with manual flexing to an angle of 45° side to side on sunflowers. Increasing airflow resulted in a 7% increase in plant height, while flexing resulted in a 22% reduction. Anten et al. (2010) also studied the effects of flexing and wind to determine if there are any differences between the two treatments on plantain. This study found that mechanical and wind treatment both resulted in a reduction in growth, though the two treatments had differing effects on morphological traits.

Most studies into the effects of mechanical treatment have focused on the responses of dicot plants, and especially horticultural crops including tomato, lettuce, and herbs. Arabidopsis has also received a lot of attention as it has been used to try to uncover underlying mechanisms of the response to mechanical stimulation. However, monocots have received far less attention. Grace, Russell and Thompson conducted a significant amount of research on the effect of environmental stresses on grasses, including wind, in the 1970's and 80's (Grace 1974, Thompson 1974, Grace 1977, Grace and Russell 1977, Russell and Grace 1978a, b, Grace and Russell 1982). Since then, only a few studies have looked at the effect of mechanical stress on grasses, such as Wang et al., (2010), who focused on the effects of mechanical stimulation on seedlings of *Lolium perenne*. This study found that brushing *Lolium* seedings resulted in shorter plants, and an increase in root/shoot ratio, but found no difference in overall plant biomass. Despite their economic importance as a staple carbohydrate source, cereals remain relatively absent from literature on

Thigmomorphogenesis and mechanical stress. The paper by Iida (2014) highlighted the effects of mechanical treatment by manual trampling on cereals including wheat and barley and found a reduction in plant height and lodging. However, these anecdotes have not been scientifically studied to verify that these effects are indeed due to the mechanical treatment.

A large portion of research on the effect of wind on wheat has focused on lodging, the permanent displacement of stems from an upright position caused by high strength winds and often exacerbated by heavy rain fall (Cleugh, Miller, and Böhm 1998). Lodging results in increased difficulty harvesting, potential for mould contamination in the lodged crop and thus a reduction in overall yield and an increase in costs to the farmer (Berry et al. 2004). Modern cereal varieties have been bred to have shorter stems and are treated with growth regulators in the field to further reduce height and thus wind loading on stems. These methods have been successful in reducing the extent and intensity of lodging events, but lodging may still occur in extreme weather events.

Wheat is an interesting crop to study the effects of mechanical stimulation on, since there is a need to better understand the effects of both wind and brushing on the growth and development of this cereal. For this experiment, the winter wheat cultivar JB Diego was chosen from the AHDB recommended list due to its common use as a control in variety trials and was the top selling wheat variety in the UK at the time of starting this investigation (Impey 2012).

In order to create a bending moment in the plants, there are several methodologies to choose from. Consequently, there is a need to evaluate several methods of treatment to ascertain

which is the most suitable for greenhouse scale experiments going forward. Wind from a domestic fan induces movement, but also increases airflow, while brushing induces bending without affecting microclimate. There is also a question here that needs to be addressed; Is the effect of mechanical stimulation created by brushing the plants equivalent to that induced by the simulated wind?

Preliminary experiments (data not included) analysed the length of exposure to simulated wind required for an effective response and found that 8 hours of constant wind at 3.5 m/s was optimal. While wind has been a popular method of treatment, brushing provides a purely mechanical treatment. Using a purpose-built rig to induce a bending moment in plant stems reflects the mechanical effects of the simulated wind. An investigation into application and timing of treatment using the rig in comparison to the fan was therefore required. A search of the literature (Garner and Björkman 1996, Telewski and Pruyn 1998) and preliminary experiments (data not included) suggested that 20 brushstrokes could be enough to create a response.

In order to induce mechanical stress in the plants, a rig was designed to apply a brushing treatment which would flex plants and ensure even and reproducible application of treatment across a tray of plants. Furthermore, it was designed to be adaptable and adjustable to treat plants of different heights, and portable so it could be moved easily and used in different locations.

Treating plants in the morning, before watering was the most suitable time for applying treatment. The leaves of plants wet from watering may adhere to the brushing bar, causing

leaves to be broken and stems to be damaged. While using a wooden bar would reduce this problem, it would be best to treat the plants prior to watering. Brushing treatment applied in the morning would also be synchronised with the start of wind treatment. This then raised several questions; does the application of the 20 brushstrokes have to be all at the same time? What if the treatment was split up into two treatments, one in the morning and another in the evening, at the same time as the fan being turned off? What would be the effect of halving the dose to 10 and just treating them in the morning be?

Aims

There were three aims of this initial experiment, firstly to test the effectiveness of the purpose-built rig and the fan – do they provide a sufficient bending motion to cause a response? The second aim is to observe the effects of two levels of treatment and treatment split between two applications. The final aim is to determine the physiological responses of young wheat plants to mechanical stimulation.

3.2 Materials and Methods

3.2.1 Preparation

150 seeds of the winter wheat variety JB Diego (Senova seeds) were sown into 3.5inch pots containing John Innes no.3 compost, 1 seed per pot, and watered daily until seeds had fully emerged. Pots containing non-germinated seedlings and seedling which had failed to grow sufficiently were discarded. Two weeks after the seedling had emerged the height of each plant was measured and the tallest and shortest were discarded, so that the remaining 100 plants were of roughly similar height (Figure 18). Plants were distributed randomly amongst five groups of 20. Each group was placed in a tray and organised five by four, then assigned a treatment – simulated wind, brushing or left untreated.

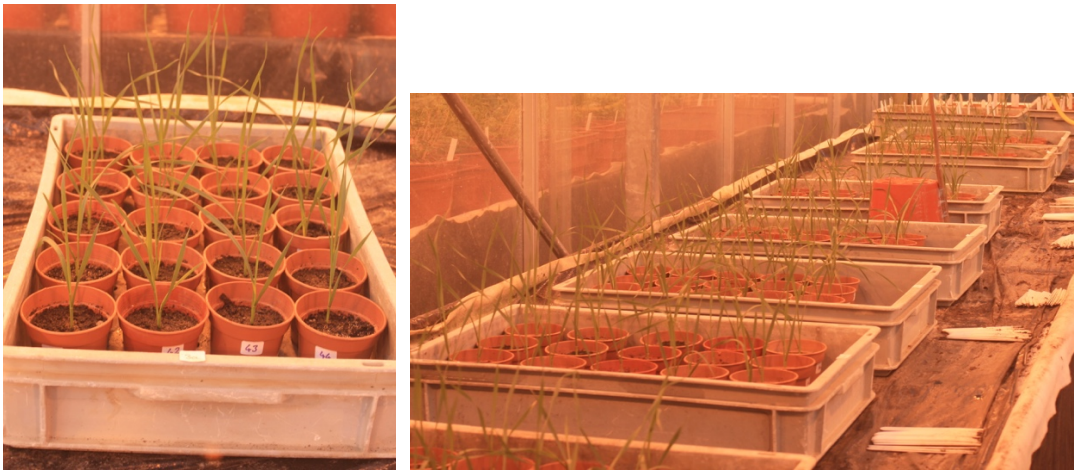


Figure 18 – Plants at 2 weeks post emergence, prior to allocation of treatments and groups. The tallest and shortest plants were discarded so that all plants were of a similar size at the start of treatment.

3.2.2 Treatment

Treatments assigned were 'Wind', or one of three levels of brushing, while one group remained untreated.

‘Wind’ treated plants were exposed to simulated wind from a domestic fan (see Figure 19) for 8 hours a day with an average wind speed of 3.5 m/s measured using an Omega instruments handheld anemometer. As the plants grew, those at the front began to shelter the ones further back, reducing the windspeed experienced by plants at the back of the group. Therefore, in an attempt to even out exposure, plants at the front were moved to the back at the end of each day, to ensure all plants in the group received even treatment from the fan.

Figure 19 - Wind treatment applied using a domestic fan.

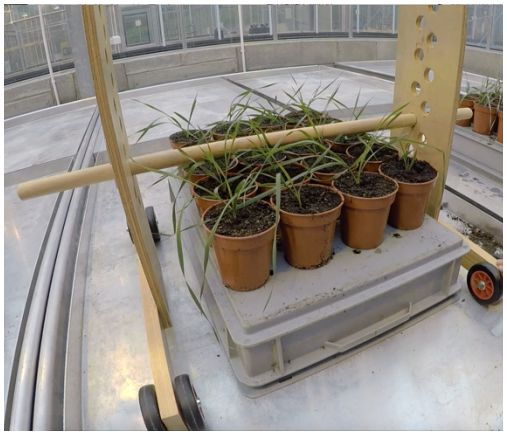


Figure 20 -Plants receiving brushing treatment.

Plants were brushed using a purpose-built rig (as shown in Figure 20), with a wooden bar set to 6 cm above the soil surface (roughly halfway up the seedlings), which was raised as the plants grew to maintain a constant bending treatment at half the canopy height. Initial trials of the brushing rig demonstrated that 20 brushstrokes were optimum for a response after 2

weeks of treatment (data not included). This experiment would analyse the effect of splitting the treatment between two 'doses' per day against one single dose of 20 brushstrokes and a single dose of just 10 brushstrokes.

Brushing treatment consisted of either:

1. 10 x 1 - Low dose treatment - Plants were treated once each morning with 10 brushstrokes from the rig. Each brushstroke involved one forward and one reverse pass of the bar over the plants – so each plant was bent both forwards and back.
2. 10 x 2 - Discontinuous brushing treatment - Plants were treated twice per day, once in the morning and once in the evening, with 10 brushstrokes each time. Therefore, plants were treated with 20 brushstrokes each day, split between 2 applications of treatment.
3. 20 x 1 - High dose treatment – Plants were treated once each morning with 20 brushstrokes.

The final group of 20 plants grew in 'static' conditions as they received no treatment and ambient greenhouse airflow measured less than 0.3 m/s. Plants were grown in a greenhouse with supplementary heating (25°c day and 10°c night) and lighting (10 hours a day) for the duration of the experiment. Treatment began 2 weeks after seedling emergence, at which point the majority of plants consisted of two fully emerged tillers. Plants were then treated daily for 4 weeks. Data was collected after both 2 weeks and 4 weeks of treatment.

3.2.3 Measurements

After two weeks of treatment, the number of fully emerged tillers of each plant was counted. Canopy height was measured from the soil surface to the top of the highest point of the plant. Leaf length and width was recorded for the two highest fully emerged leaves on the main tiller. These measurements were also repeated after 4 weeks of treatment. After phenotypic measurements were taken at the end of 4 weeks of treatment, all plants were destructively harvested. Plants were cut at their base (at or just slightly under the soil surface) and separated into above and below ground material. Fresh above ground material was weighed (fresh weight), then dried by placing the samples in an oven at 60°c for 48 hours and weighed again (dry weight). The moisture content was calculated on a fresh weight basis using the following equation: moisture content % = [(fresh weight (g) – dry weight (g)) / fresh weight (g)] * 100%. For below ground biomass measurements, 5 plants from each treatment were randomly selected. The roots from the selected plants were separated from the soil by firstly gently breaking up the soil mass, then soaking the soil/root mass in water, to loosen soil from the roots. The remaining soil/root mass was placed on a fine sieve, under a running tap, and gently agitated to loosen and remove the remaining soil. Once the roots had been cleaned, excess water was removed by placing them on a paper towel, and samples were dried in an oven at 60°c for 48 hours and dry weight was determined

3.2.4 Statistics

Data was first checked for normal distribution and homogeneity of variances. Initially, an analysis of Variance test was conducted using SPSS statistics software package (IBM corp. Released 2016. IBM SPSS Statistics for Windows, version 24.0. Armonk NY: IBM corp.), then if

ANOVA indicated that results were significant, a post-hoc test using Tukey's HSD and Dunnett t were conducted.

3.3 Results

3.3.1 Height

Plant height was measured as the distance from the soil surface to the highest point of the plant. After two weeks of treatment, there was a notable and significant (ANOVA $p<0.001$) reduction in plant height between treated plants and controls (Figure 21). Brushing appears to have a greater effect on height than wind treatment, with 10x2 treatment having the greatest effect. The effect of brushing on plant height was highly significant ($p<0.001$ for all brushing treatments), while the fan treatment also had a significant effect ($p<0.005$).

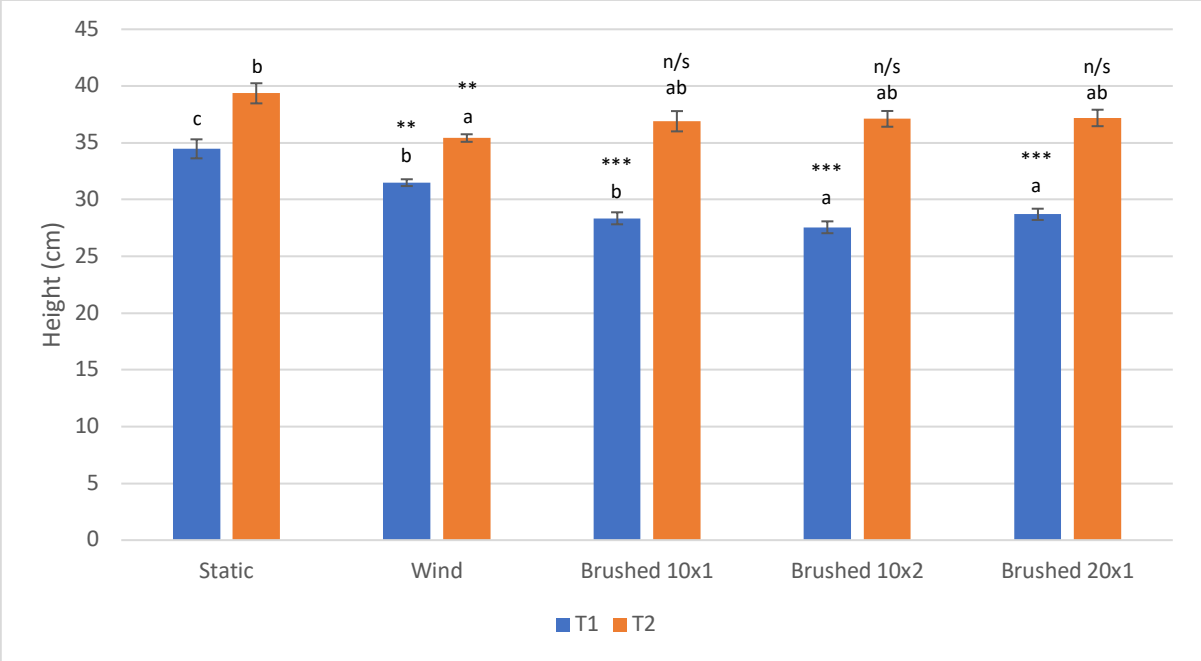


Figure 21 Plant height after two (T1) and four (T2) weeks of treatment. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

After a further 2 weeks of treatment, the difference between treated and untreated plants was much reduced. Again, there was a significant difference between treated and untreated

plants (ANOVA p<0.05), but this time wind treated plants had the greatest reduction in height while there was no difference between brushing treatments.

3.3.2 Tillers

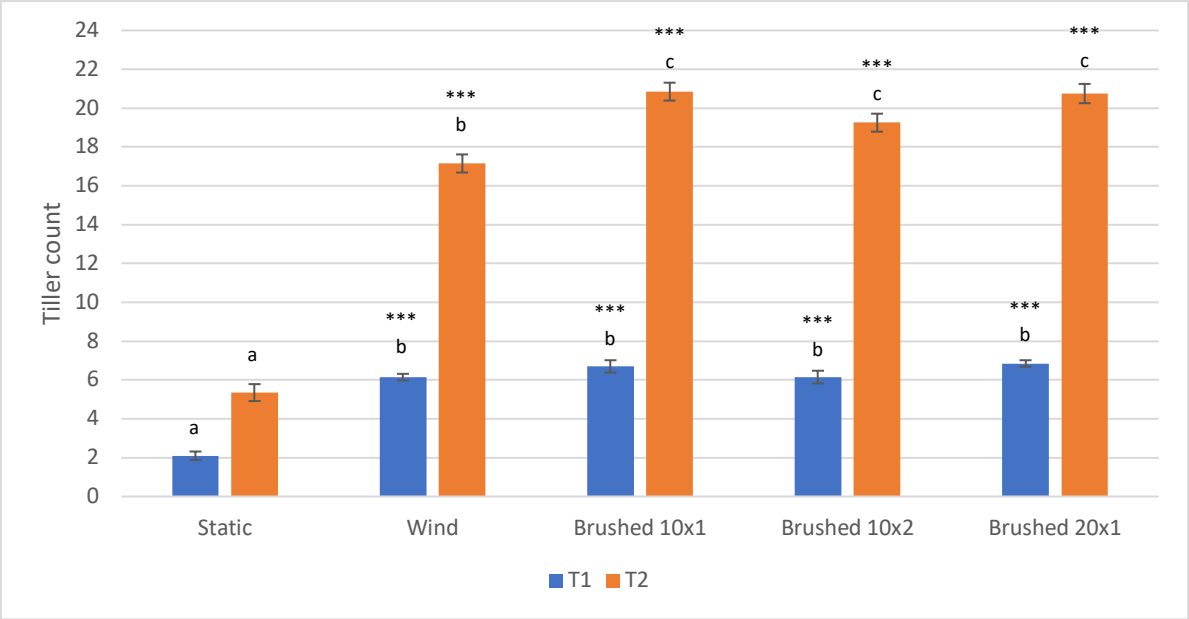


Figure 22- Average number of tillers per plant after two (T1) and four (T2) weeks of treatment. Error bars indicate standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

Treated plants had significantly more tillers than untreated plants after the first two weeks of treatment (ANOVA p<0.001). Untreated plants had an average of 2 tillers, while treated plants had three times as many (6 on average). There was no difference in the number of tillers between treatments, as seen in Figure 22.

After a further two weeks of treatment, the difference in tiller number between treated and untreated plants became much more pronounced (ANOVA p<0.001). While untreated plants had an average of 5 tillers, treated plants had between 17 and 21 tillers per plant. The difference between treated plants also became more obvious after four weeks of treatment.

3.3.3 Leaves

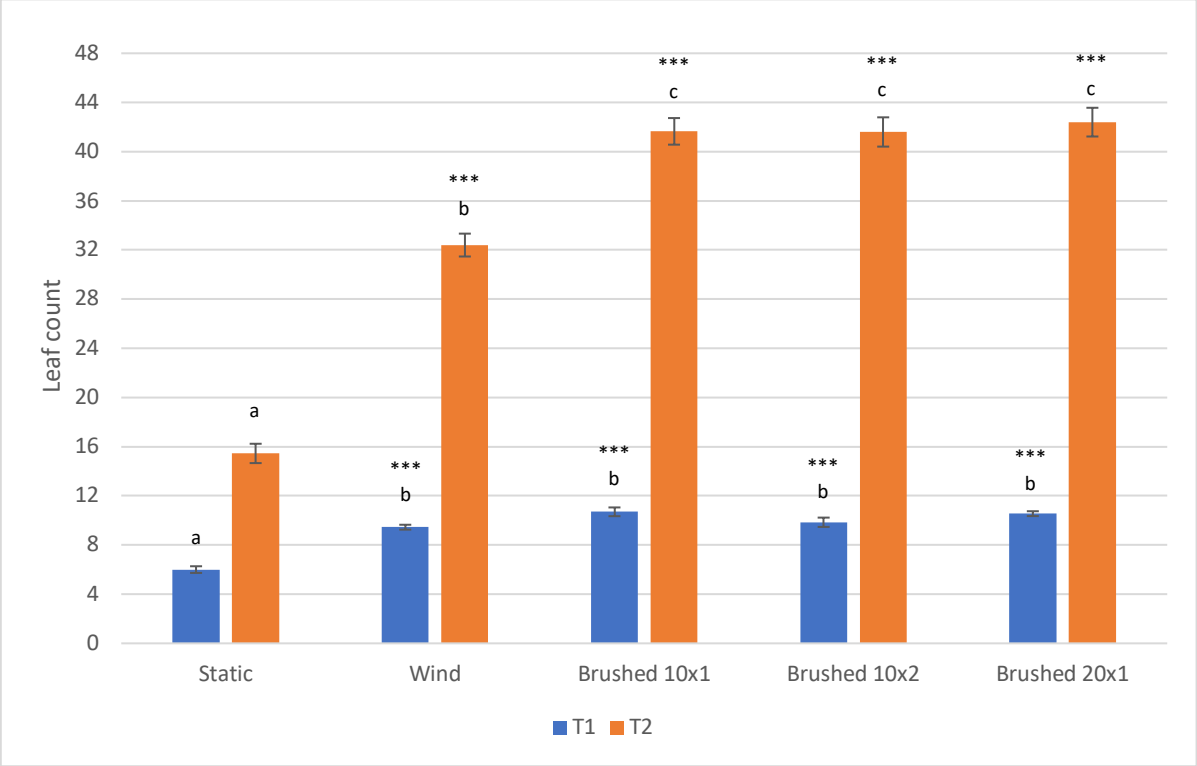


Figure 23 - Average number of leaves per plant after two (T1) and four (T2) weeks of treatment. Error bars indicate standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

After two weeks of treatment, treated plants had significantly more leaves than untreated plants (ANOVA p<0.001) (Figure 23). While wind treated plants had the fewest number of leaves per treated plant (9.5), there was no difference overall between treatments with 10x1 brushed plants having the most leaves at 10.7 on average. After a total of four weeks of treatment, the difference between treated and untreated plants became even more obvious, also shown in Figure 23. While static plants had an average of 15.5 leaves per plant, treated plants had between 32.4 and 42.4 leaves per plant. Wind was the least effective treatment, however there was little difference in effect between the three brushing treatments.

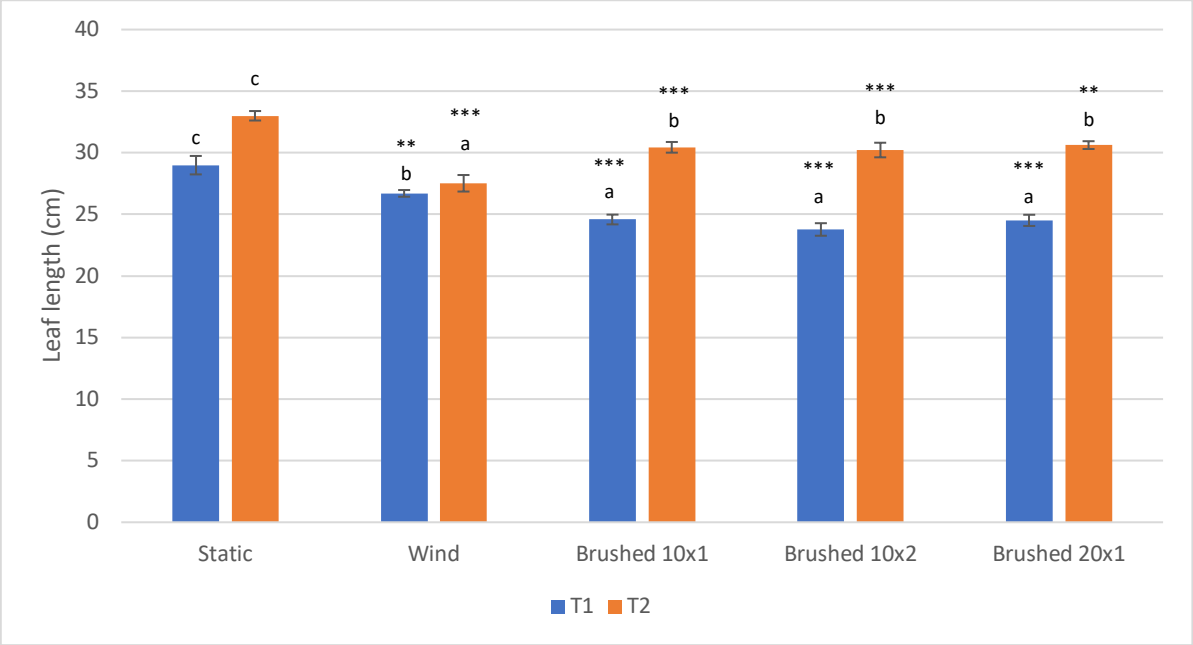


Figure 24 - Average length of the topmost leaf on the main tiller after two (T1) and four (T2) weeks of treatment. Error bars indicate standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

After two weeks of treatment, the length of the topmost leaf on the main tiller was measured (results shown in Figure 24). The topmost leaf of treated plants was significantly shorter than those of statically grown plants (ANOVA $p<0.001$). Brushing treatments resulted in 15-18% shorter leaves than controls, where-as the leaves of wind treated plants were 8% shorter. 10x2 treated plants had the shortest leaves of all treatments. After a further two weeks of treatment leaf measurements were repeated. This time wind treated plants had the shortest leaves, also shown in Figure 24. All three brushing treatments resulted in smaller leaves than the static plants, there was little difference between the treatments.

3.3.4 Biomass

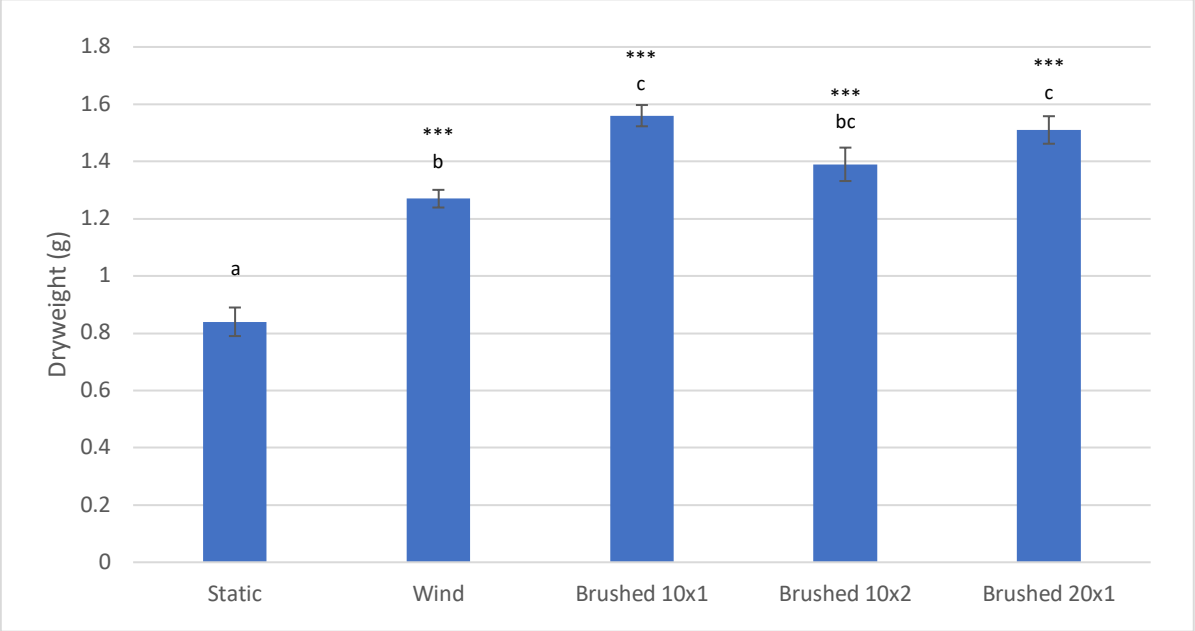


Figure 25 - Average plant above ground dry weight (Biomass) after four weeks of treatment. Error bars indicate standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

To determine if exposure to the experimental treatments impacted on above ground and below ground biomass accumulation, plants were destructively harvested after 4 weeks of treatment, then oven dried to determine biomass. Mechanical treatment leads to an increase in biomass accumulation above ground (Figure 25). While all treatments had significantly greater biomass than untreated plants ($p<0.001$), 10x1 treatment had the greatest effect and wind treatment the least.

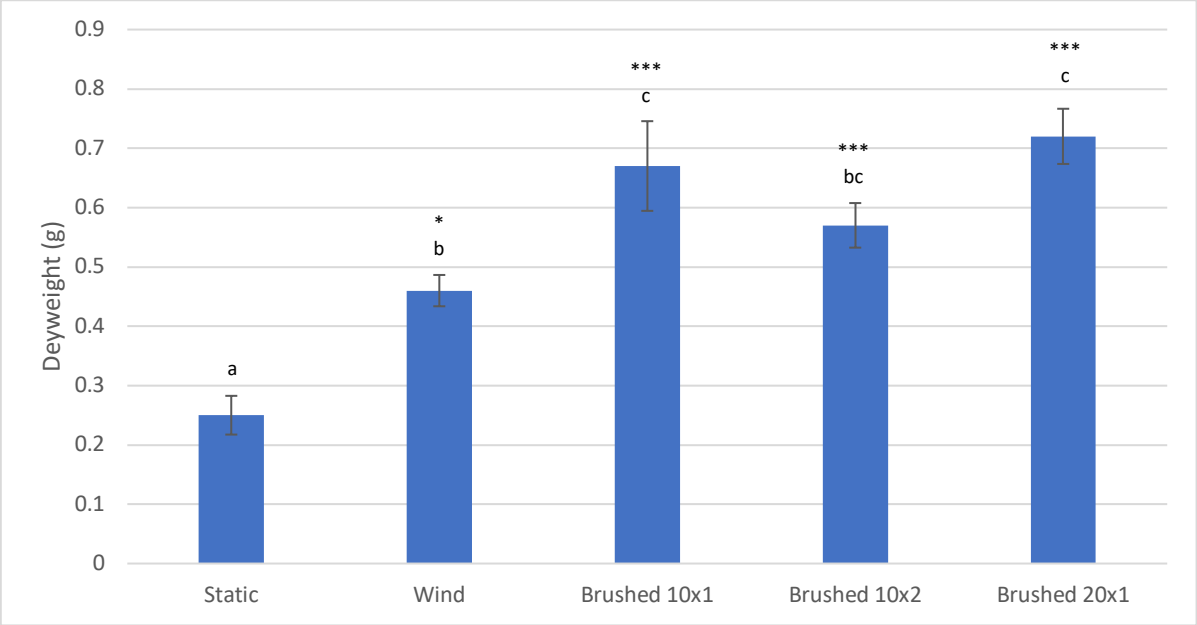


Figure 26 - Below ground biomass (root dry weight) after four weeks of treatment. Error bars indicate standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=5.

After 4 weeks of treatment, 5 plants form each treatment were randomly selected, their roots separated from the soil, then dried and weighed to determine below ground biomass. Below ground biomass showed a similar trend to the above ground biomass, with all treatments having a significant increase in biomass ($p>0.001$). Splitting treatment between two applications each day was similar to the effect of wind, shown in Figure 26. The high intensity treatment, 20x1 which involved 20 brushstrokes applied once per day had the greatest effect on below ground biomass, indicating that plants increase response with increasing intensity of treatment. It appears that 20x1 brushing treatment had a greater effect on below ground than above ground biomass, compared with other treatments, while the other treatments followed the same pattern of allocation as before.

3.4 Discussion

3.4.1 Plant Height

Results indicate that wheat is sensitive to mechanical perturbation, as also noted by Steucek and Gordon (1975), and Crook and Ennos (1996). After two weeks of treatment plants were significantly shorter than controls across all treatments. The most frequently reported response to mechanical treatment is indeed a reduction in height seen both in forage grasses, including *Lolium* and *Festuca* (Russell and Grace 1978a, Grace and Russell 1977, Wang et al. 2010), as well as dicots such as sunflower, Arabidopsis, tomato and pine trees (Paul-Victor and Rowe 2010, Garner and Björkman 1996, Smith and Ennos 2003). After a total of four weeks of treatment, the difference between treated and untreated plants became much smaller. Crook and Ennos (1996) found no difference in the stem height of wheat plants that had been supported, compared with plants that could sway freely in the wind, after two months of growth. In comparison, Whitehead and Luti (1962), found a significant difference in the height of Maize plants after 40 days of wind treatment, while Goodman and Ennos (1996) found that 12 weeks of flexing treatment had a no effect on plant height. Zhao et al. (2018) found that 2 weeks of treatment had no significant effect on height, but extending treatment duration to five weeks lead to a significant reduction in shoot height for treated plants. The lack of difference in response between treated and untreated plants at four weeks may be due to the variety used for this experiment. The seedlings of JB Diego used here did not undergo vernalisation, therefore preventing them from initiating flowering. In vernalised plants, flowering stems should be starting to form by four weeks, whereas in this experiment the plants remained in a purely vegetative growth phase. Response at 4 weeks was thus likely limited because growth and development of the plants had slowed. Russell and Grace (1978a), mention that responses of *lolium perenne* to wind may have been lessened as plants

became pot-bound. This may be the case with these plants as a significant decrease in height would also be expected at four weeks. Therefore, further experiments will need to use larger pots to ensure wheat plants do not become pot bound.

3.4.2 Tillers

Treated plants had more tillers after both two and four weeks of treatment. The effect of mechanical stress on tillering of forage grasses and cereals has received very little attention with only a few papers revealing any effect on tillers. Both Russell and Grace (1978a), and Wang et al. (2010), found no difference in tiller numbers after mechanical treatment of *lolium perenne. Czepak et al. (2019)* did not find any change in tillering following mechanical treatment. Crook and Ennos (1996) also found no difference in tiller numbers between supported and unsupported wheat plants exposed to wind, though this was measured at a later growth stage than in the present study. However, Zhao et al. (2018) found an increase in tillering of 23.25% and 21.36% following R30 and R60 doses of rubbing treatment in Rice plants. Additionally, Iida (2014) mentions that mechanical treatment of wheat and barley seedlings has been seen to increase tillering. During normal growth, contact form the overlying leaf sheath suppresses tiller bud growth. It is likely that brushing displaces or moves the leaf sheath, which stimulates or encourages growth of the tiller bud (Liu and Finlayson 2019), though this mechanism is not yet fully understood and more research is needed.

3.4.3 Leaves

Treated plants had more leaves than untreated plants, which is due to an increase in the number of tillers produced by treated plants. Russel and Grace (1978a) found a 21% reduction in the number of leaves per tiller in wind exposed *Lolium perenne*. Russell and Grace (1979)

also described wind treated *festuca arundinaceae* plants as 'less leafy' than untreated plants. However, few other experiments have commented on any changes in leaf count, instead using only above ground biomass as an indicator of changes in foliage. Russell and Grace (1979), attributes a small amount (10%) of reduction in leafiness to loss through damage in wind exposed plans (10 m/s). These wind speeds were significantly greater than those used in the present study, so less damage would be expected. It is much more likely that reductions in leaf number are due to responses to mechanical treatment rather than damage. Total number of leaves of wind treated plants was slightly less than for brushed plants. This is consistent with results found by Anten et al. (2010).

A significant decrease in leaf length was observed after two weeks of treatment, but this became less noticeable after four weeks. Russell and Grace (1979) observed that leaf extension decreased with increasing wind speeds and higher wind speeds resulted in shorter leaves. Both Grace and Russell (1977) found wind reduced leaf length of *Festuca arundinacea* by 12%. Whitehead and Luti (1962), found wind treatment reduced Maize leaf area by 48%. A reduction in leaf length in wind exposed plants could be attributed to damage as leaves flutter and collide. However, the top leaf, as measured here, is the youngest/most recently fully emerged leaf on the main tiller, therefore is unlikely to have suffered much damage. Top leaf length is probably not the most representative measurement for analysing the effects of mechanical treatment. Most studies look at the length of multiple leaves.

3.4.4 Biomass

Both above and below ground biomass increased in response to treatment. These results differ to those found in other studies. Niklas (1998) found that mechanical stimulation of

shepherds purse plants resulted in an increase in root biomass. Anten, Casado-Garcia and Nagashima (2005) found that flexed plants allocated a larger fraction of mass to roots than untreated plants, but a second experiment found no difference in mass allocation. Flexing Maize plants resulted in an increase of 19% in sunflower and 22% in maize plants in an experiment by Goodman and Ennos (1996). However, other experiments have observed a decrease in root biomass following mechanical treatment. Whitehead and Luti (1962) noted a 30-39% reduction in root biomass of maze plants, accompanied with a reduction in above ground biomass following wind treatment. Similarly, Anten et al. (2010) found a 47% reduction in root biomass after wind treatment, and a 62% reduction following brushing treatment of plantain plants. Russell and Grace (1978a) found that *Lolium* above ground biomass was significantly reduced after 67 days of wind treatment, but the difference between treated plants and controls was not significant after 120 days of treatment. Wang et al., (2010) found there was no overall change in total biomass, although there was an increase in root biomass, which indicates a reciprocal reduction in above ground biomass in brushed *Lolium*. Zhao *et al.* (2018) found that only high intensity (R90) rubbing resulted in significant reductions in above ground biomass, while the lower intensity treatments (R30 and R60) were more similar to controls. Only low R30 treatment affected below ground biomass, resulting in higher root biomass compared with controls, while R60 and R90 treatments had no effect.

3.4.5 Treatments

For all the traits measured, simulated wind from the fan had, essentially, the same overall effect as brushing, though the extent and impact of the different treatments varied. Smith and Ennos (2003), noted that wind exposure (without flexing) had opposite effects on

sunflower plants to flexing treatment. Wind treated plants (without flexing) were 7% taller than controls, while flexed plants were 22% smaller. When wind was combined with flexing, the effects were closer to flexing without wind, but airflow was obviously having additional (and opposite) effects to the mechanical treatment. Anten *et al.* (2010) also found that in some cases airflow had opposite effects to mechanical treatment. These studies indicate that the effects of mechanical stimulation on plant growth and development cannot simply be extrapolated to the effects of wind, as has been suggested by others (Niklas 1998, Anten, Casado-Garcia, and Nagashima 2005). Although, the purely mechanical aspect of wind on plants can be directly related to alternative forms of mechanical stimulation.

Wind has significant effects on the microclimate of plants, with airflow affecting both transpiration and leaf temperature (Anten et al. 2010). Some responses to wind may actually be a reaction to limit water loss and thus prevent dehydration, while others may be attributed to mechanical treatment, making the two effects of wind hard to separate. Nevertheless, Anten et al. (2010) suggests that the extent to which plants respond to the two effects may be dependent on their growth form, with taller plants (e.g. sunflower) needing to respond to the mechanical element and low-growing plants (e.g. plantain) responding to reduce water loss.

In this experiment, the 'static' plants could be considered the odd one out and wind treatment more like a control. In the natural environment, plants are exposed to wind almost constantly at an ambient level. Growing without this mechanical stimulus is an un-natural environment for plant growth. However, growing plants under controlled conditions and using a greenhouse allows conditions like light, nutrients, and temperature to be manipulated so that

the effects of a particular treatment can be evaluated in isolation. In this case, by providing an environment free of mechanical stimulation, the effect of applying it can be examined closely.

A few limitations in the methodology used in this study have been considered. Firstly, mechanical forces by each of the treatments was not quantified, because the plants were too small and lacked stems to attach a strain gauge to, therefore it is unknown whether wind treated plants received the same amount of mechanical force as the brushed plants. Secondly, the treatment using a domestic size fan in greenhouse not sufficient for treating more mature wheat plants. From about two weeks, plants became very bushy, which resulted in a sheltering effect on plants further from the fan. Despite rotating rows from back to front daily to even out the treatment, plants at the back of the block received significantly reduced mechanical treatment compared with those at the front. Limitations due to the size of the greenhouse prevent using a larger fan, which could also have an impact on the untreated plants growing in the same greenhouse. However, results indicate that brushing had very similar effects to wind treatment, i.e. reduction in height at T1 and no change at T2, increased tillering, increased leaf count and increased above and below ground biomass.

This experiment evaluated two different methods for applying mechanical treatment wind and brushing. Further experiments will focus on the use of brushing as mechanical treatment due to the absence of potential confounding effects from increasing airflow around the plants. Additionally, towards the end of the treatment period, the fan used was not able to create sufficient motion in larger plants and scaling up the size of the fan is not possible in the greenhouse space available. Brushing provides a more controlled application of mechanical

stimulation and more pronounced effects, as found in this study. The next step is to look more closely at the application of brushing treatment and evaluate the effect of a single application of treatment as well as a range of treatment doses to identify the sensitivity of wheat to mechanical stimulation.

Chapter 4

4 Investigating the effect of treatment dose on the growth and development of wheat

4.1 Introduction

Chapter three established that wheat responds very similar to mechanical treatment by brushing when compared to exposure to simulated wind. In addition, a successful method of brushing multiple wheat plants at the same time was developed. The next step is to further refine the level of treatment that creates a response and identify if there is a threshold or a level before which the plant does not respond to the mechanical treatment.

In the paper “Mugifumi” by Iida (2014), the cultural practice of mugifumi was described; the process involves walking or treading on the seedlings of cereal crops in the field in order to encourage growth and improve yield. Though studies have been conducted in the past, none are currently available in English. However, Iida sites a Japanese study that demonstrates that the practice increases tillering, root spreading, number of spikes per plant, and also grain weight. Both Berry et al. (2004), and Scott (2005) noted reductions in the lodging of plants

that lined tramways in fields cropped with cereals and suggested that this may be due to movement of machinery up and down the field. Wheat plants lodge in two ways; Stem lodging involves a bending or breaking of the lower culm internodes, whereas root lodging involves straight and intact culms which become displaced and lean from the crown and some disturbance to the root system (Pinthus 1974). Rolling crops is also regularly used as a management practice to compact soil and reduce root lodging, though it has also been noted that rolling may shorten crop height thus reducing both stem and root lodging (Harris 1986, Berry et al. 2002, Berry et al. 2004).

Previous studies on the dose-response of plants to mechanical stress have mostly focused on dicots, like most of the research associated with thigmomorphogenesis and plant responses to mechanical stress. Telewski and Prydin (1998) studied the effects of repeated flexing (0, 5, 10, 20, 40, and 80 flexures) on the growth of *Ulmus americana*. Both plant height and leaf area were reduced following treatment and the effect was roughly linear with increasing number of flexures. Stem diameter increased up to 40 flexures, though there was a reduction following 80 flexures. Jaffe (1980) measured stem elongation in bean plants following manual rubbing treatment of the stems with doses ranging from 0 to 20 rubs. They found a logarithmic relationship between rubbing force and elongation, and also noted that the sensory system became saturated after just two rubs. However, some recent studies have looked at the effect on monocots; Zhao et al. (2018) investigated the effect of varying levels of rubbing treatment on rice plants. They observed the effects of rubbing rice stems 0, 30, 60, and 90 times per day for 35 days. The study found no difference in shoot height, but elongation rates were significantly reduced for treated plants compared with controls. The length of the second internode was also significantly reduced for treated plants compared

with controls, while all three treatments resulted in a significant increase in stem width. Both R30 and R60 treated plants had significantly more tillers than controls, though R30 plants had 1.89% more. Low dose treatment also significantly improved chlorophyll content, transpiration rate, nutrient content, and soluble protein content, more so than the other treatments. The paper concluded that overall, low doses of treatment had the greatest effects on the parameters measured. It would be interesting to undertake a similar study to determine the effect of a range of doses of mechanical treatment on the growth and development of wheat.

Previous studies indicate that there are differences in the way plants respond to mechanical treatment and the airflow associated with wind (Grace 1977, Grace and Russell 1977, Anten et al. 2010). In order to study the effects of mechanical treatment in isolation, it is therefore best to grow plants in a controlled environment, such as a greenhouse, where airflow can be kept to a minimum.

In the previous chapter, while both 10 and 20 repetitions of brushing produced an effective response, the higher dose was often more effective, therefore the next experiment will include 20 brushstrokes as the maximum dose for this dose-response experiment. Treatments will comprise 1, 3, 6, 9, 12, 15 and 20 brushing repetitions in order to develop a dose-response curve for multiple plant measurements, both phenotypic and mechanical, and to identify a threshold for the responses. While the previous experiment focused on the effects on young plants, this chapter will look at the effect immediately after treatment as well as lasting effects at later stages of growth and development. Using mature plants there is an

opportunity to look at the mechanical properties of stems which change as the plant grows and senesces. Furthermore, grain yield is an important agronomic trait of wheat.

The aim of this experiment is to measure plant phenotypic traits in response to increasing doses of brushing, and the effects of treatment on main tiller mechanical properties and grain yield.

4.2 Materials and methods

4.2.1 Plants, treatments and measurements

350 seeds of the spring wheat variety Mulika (Senova Seeds) were planted into 5-inch plastic pots, 1 seed per pot, containing John Innes number 3 compost on the 6th of July 2017. Seeds were well watered and placed in a greenhouse with supplementary heating (20°C day / 10°C night) and lighting (10 hours, 8am-6pm). 2 weeks after planting the seeds, the plants were inspected and those with either poor or excessive growth were removed, as well as pots containing seeds that did not germinate. Remaining plants were randomly assigned to a group and each group assigned to a treatment. Each group (rep) consisted of 8 plants, with 3 groups per treatment, therefore totalling 24 plants per treatment. Treatment involved brushing the plants with a purpose-built rig, as described in chapter 3, but the number of brushstrokes applied each day were 1, 3, 6, 9, 12, 15 and 20. An additional 24 plants, split into three groups received no treatment. Treatment began two weeks after seedling emergence and treatments were applied once per day, each morning, for four weeks (28 days). Plants were watered after the application of treatment, and again in the evening, by filling the trays the plants sat in so as to not disturb aerial plant parts, and to prevent damage when applying treatment. Once plants consisted of three tillers, a small piece of brightly coloured wool was tied to the initial tiller of each plant, to mark it as the main tiller.

After 4 weeks of treatment (timepoint 1, T1) plants had reached growth stage 31, and phenotypic data was recorded, including plant height and the number of tillers produced per plant. Plants were then left to continue growing and watered daily until they had finished flowering (GS69), when additional data was collected. Phenotypic data collected after flowering had finished (timepoint 2, T2) included main tiller height to top of spike, number of

flowering spikes, main tiller internode lengths and diameter, and flag leaf length and width. Nine plants from each treatment (three from each group) were randomly selected and destructively harvested. From the selected plants, the main tiller was removed, and mechanical tests were carried out immediately, so as to reduce the effect of wilt on the mechanical properties of the stem. Mechanical tests were fully described in chapter 2.

Remaining plants were left to grow until they reached maturity and under the warm greenhouse conditions became completely senesced. The number of flowering spikes was counted again, and the length of each spike was recorded. The spikes on each plant were then labelled, cut from the stem, and weighed to determine the weight of the main tiller spike and all spikes from each plant. Remaining above plant material was cut at the base with a sharp knife and each plant was weighed to determine the biomass of each plant. The main tiller was also weighed separately. Main tillers were then striped of the leaves and leaf sheath, processed for mechanical tests.

Stem mechanical properties including Young's modulus and bending strength were determined as explained in chapter 2.

4.2.2 Statistical methods

Data was checked for normal distribution and homogeneity of variance, followed by either Analysis of Variance (ANOVA) or t-tests. If ANOVA indicated that the data was significant, a Tukeys HSD post-hoc test was conducted. Statistical tests were performed using the SPSS statistical software package (IBM Corp. Released 2016. IBM SPSS Statistics for Mac, version 24.0. Armonk, NY: IBM Corp).

4.3 Results

4.3.1 T1 Height

Treatment had a very noticeable effect on height, with a distinct reduction in height as the number of brushstrokes applied per day increased (Figure 27 and Table 1). The application of just one brushstroke per day for four weeks reduced height by 171 mm, compared with untreated plants. Plants that received 1 brushstroke were 24% shorter than those that were untreated but were 39% taller than those that received 20 brushstrokes. All treatments had a significant effect on height (ANOVA $p<0.001$) compared with untreated plants. Post-hoc test results shown in.

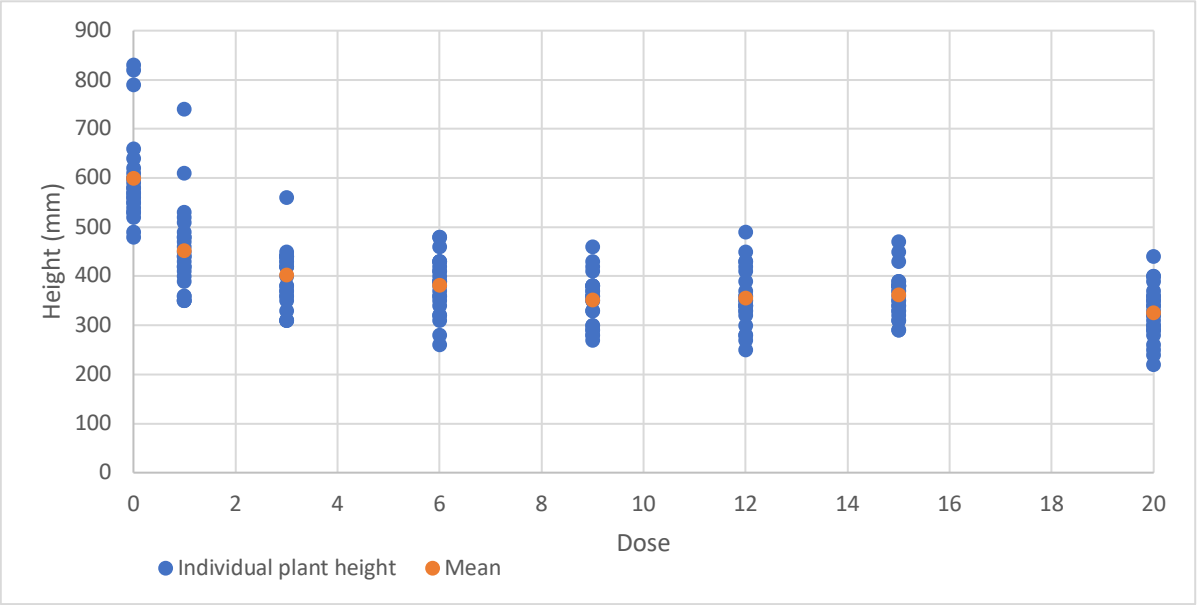


Figure 27 - Plant height following four weeks of treatment (T1) at a range of doses – 0, 1, 3, 6, 9, 12, 15, and 20 brushstrokes per day.

Table 1 - Mean plant heights following four weeks of treatment (T1) at a range of doses – 0, 1, 3, 6, 9, 12, 15, and 20 brushstrokes per day. Stars indicates the data is significantly different to controls according to a Dunnett t test. Measurements with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$).

Treatment	Height (mm)	n	Std dev	SEM		
0	598.8	24	98.51	20.11	a	
1	452.5	24	89.60	18.29	b	***
3	402.2	23	55.76	11.63	bc	***
6	382.1	24	58.16	11.87	cd	***
9	351.3	24	51.44	10.50	cd	***
12	355.4	24	60.14	12.28	cd	***
15	362.1	24	45.78	9.34	cd	***
20	325.4	24	54.77	11.18	d	***

4.3.2 T2 Main Tiller height

Main tiller height was recorded at the end of flowering and measured from soil surface to the tip of the flower. Treated plants were shorter than untreated plants, but the trend was not as clear as seen at the end of treatment (T1), though there was still a downward trend in height as the number of brushstrokes increase (Figure 28). However, while 1 brushstroke resulted in an average reduction of 27 mm and 6 brushstrokes 27 mm, 3 brushstrokes reduced height by 99 mm compared with untreated plants (Table x2. Increasing the number of brushstrokes from 9 onwards resulted in a downwards trend in height, with 15 brushstrokes reducing height by 73.0 mm. However, 20 brushstrokes appear to be less effective than both 12 and 15, with only a reduction of 63.7 mm compared with untreated plants. 1, 6, and 9 brushstrokes did not have a significant effect on height, while 3 brushstrokes had a highly significant effect (Dunnett t $p<0.001$) followed by 15 brushstrokes ($p<0.01$) and then both 12 and 20 brushstrokes ($p<0.05$).

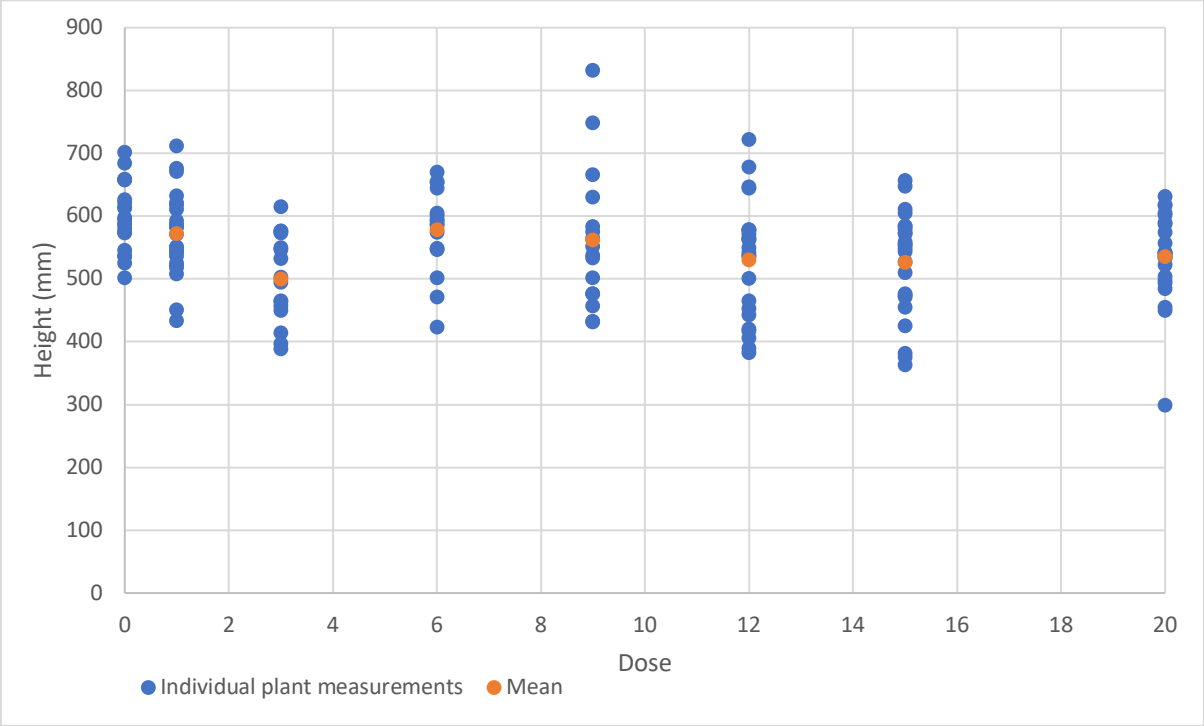


Figure 28 Main tiller height at the end of flowering (T2) following earlier treatment of increasing numbers of brushstrokes per day (dose).

Table 2 - Mean main tiller height at the end of flowering (T2) following earlier treatment of increasing numbers of brushstrokes per day (dose). Stars indicates the data is significantly different to controls according to a Dunnett t test. Measurements with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$).

Treatment	Height (mm)	n	S.D.	SEM		
0	598.8	24	56.14	11.459		b
1	571.9	24	65.96	13.464	n/s	ab
3	500.2	23	69.79	14.553	***	a
6	572.4	24	65.84	13.440	n/s	ab
9	562.4	24	111.96	22.854	n/s	ab
12	530.5	24	92.84	18.951	*	ab
15	526.3	24	82.53	16.846	**	ab
20	535.6	24	71.05	14.503	*	ab

4.3.3 Internode length (T2)

All of the main tillers measured consisted of 4 internodes which have been numbered from bottom (1) to top (4). Treatment had a highly significant effect on the lowest two internodes (ANOVA $p<0.001$), resulting in a reduction in length compared with plants that did not receive any treatment. There was some reduction in the length of internode 3, but treatment resulted

in an increase in the length of internode 4 – though this did not offset the reduction in length of the other internodes, therefore leading to an overall decrease in plant height.

For all but one treatment, treated plants had a highly significantly shorter base internode (Dunnett t $p<0.001$) compared with untreated plants (Figure 29), however, 6 brushstrokes was slightly less effective ($p<0.01$). The base internode of untreated plants was 53.45 mm on average, with 1 brushstroke reducing length by 23.34 mm and 20 brushstrokes by 30.87 mm, while plants that received 6 brushstrokes were 22.26 mm shorter.

The second internode of treated plants was also shorter than untreated (Figure 29), with the treatment having a significant effect on height across all the treatments (Dunnett t $p<0.001$). On average, the second internode of untreated plants was 95.17 mm while plants that received 1 brushstroke were 69.63 mm long and 20 brushstrokes resulted in an average length of 56.92 mm. Of all the treatments, 3 brushstrokes had the greatest effect, with a reduction of 45.23 mm and 1 brushstroke was the least effective. A Tukey HSD post-hoc indicated that there was no significant difference in internode 2 length of plants that received more than 3 brushstrokes.

There was a reduction in the length of internode three after the application of brushing (Figure 29), however, plants that received 6 brushstrokes were 6.08 mm longer than untreated plants. Overall, treatment dis not have a significant effect on the length of the third internode.

The top internode of treated plants was longer than for untreated plants which were 205.33 mm long on average (Figure 29), though treatment did not have a significant effect on the length of internode 4 overall.

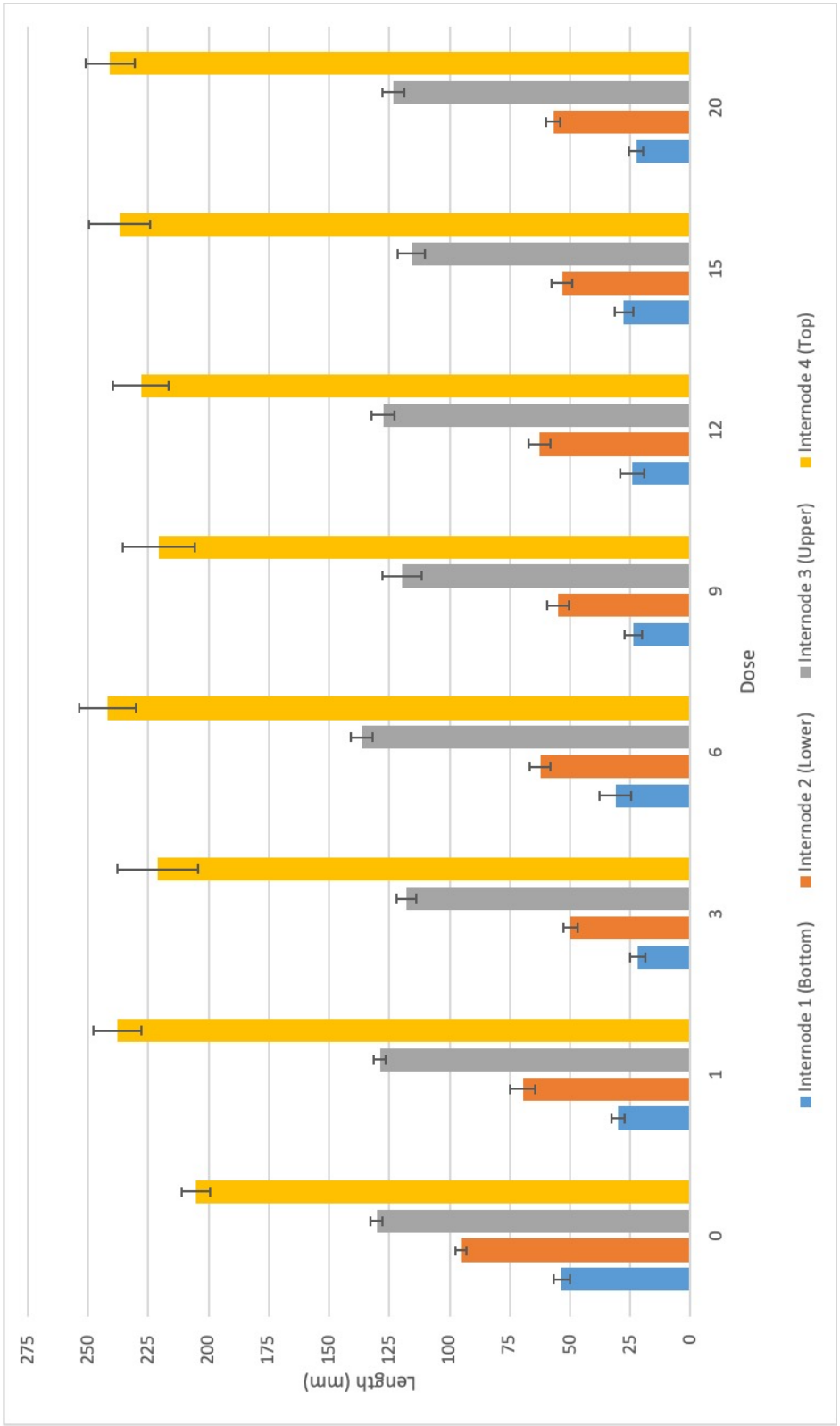


Figure 29 - Main tiller internode lengths at the end of flowering (T2). Internode numbered from bottom (1) to top (4) in response to increasing doses of brushing treatment. Error bars indicate ±1 Standard error of the mean

4.3.4 Internode diameter

Treatment generally reduced internode diameter (Figure 30), though there was no particular trend relating to increasing the dose of treatment. Treatment appears to have had very little effect on the base internode, but a much greater effect on the third internode.

All brushing treatments, except for 12 brushstrokes, resulted in a reduction in the diameter of internode 1. 12 brushstrokes resulted in an increase of 0.06 mm compared with control stems which were 2.79 mm wide on average (Figure 30). Plants that received 3 brushstrokes had the narrowest first internode, 2.40 mm on average. Only 3 brushstrokes had a significant effect, Dunnett t $p<0.01$.

All treatments resulted in a narrower second internode compared with controls (Figure 30). Again, 3 brushstrokes resulted in the greatest effect, with an average diameter of 2.99 mm compared with 3.36 mm for controls. 3 brushstrokes had a significant effect on diameter of the second internode, Dunnett t $p<0.01$, as did 20 brushstrokes, $p<0.05$.

The diameter of the third internode across all treatments was reduced compared with controls (Figure 30). One brushstroke had the least effect, with an average diameter of 3.67 mm compared with 3.91 mm for controls. This difference was not significant. Plants that received 15 brushstrokes had the narrowest stems. Excluding the lowest dose, all other treatments had a significant effect on the diameter of internode 6 and 12 brushstrokes $p<0.01$ and 3, 9, 15, and 20 $p<0.001$ (Dunnett t).

One brushstroke resulted in wider stems than controls, though all other treatments resulted in narrower stems (Figure 30). Plants that received 3, 6, or 9 brushstrokes had a narrower fourth internode than plants that received greater doses of treatment, 12, 15, or 20 brushstrokes. Moreover, out of all treatments, 20 brushstrokes resulted in an average forth internode diameter very close to that of controls, only 0.04 mm difference. Only 9 brushstrokes had a significant effect, Dunnett t $p<0.05$.

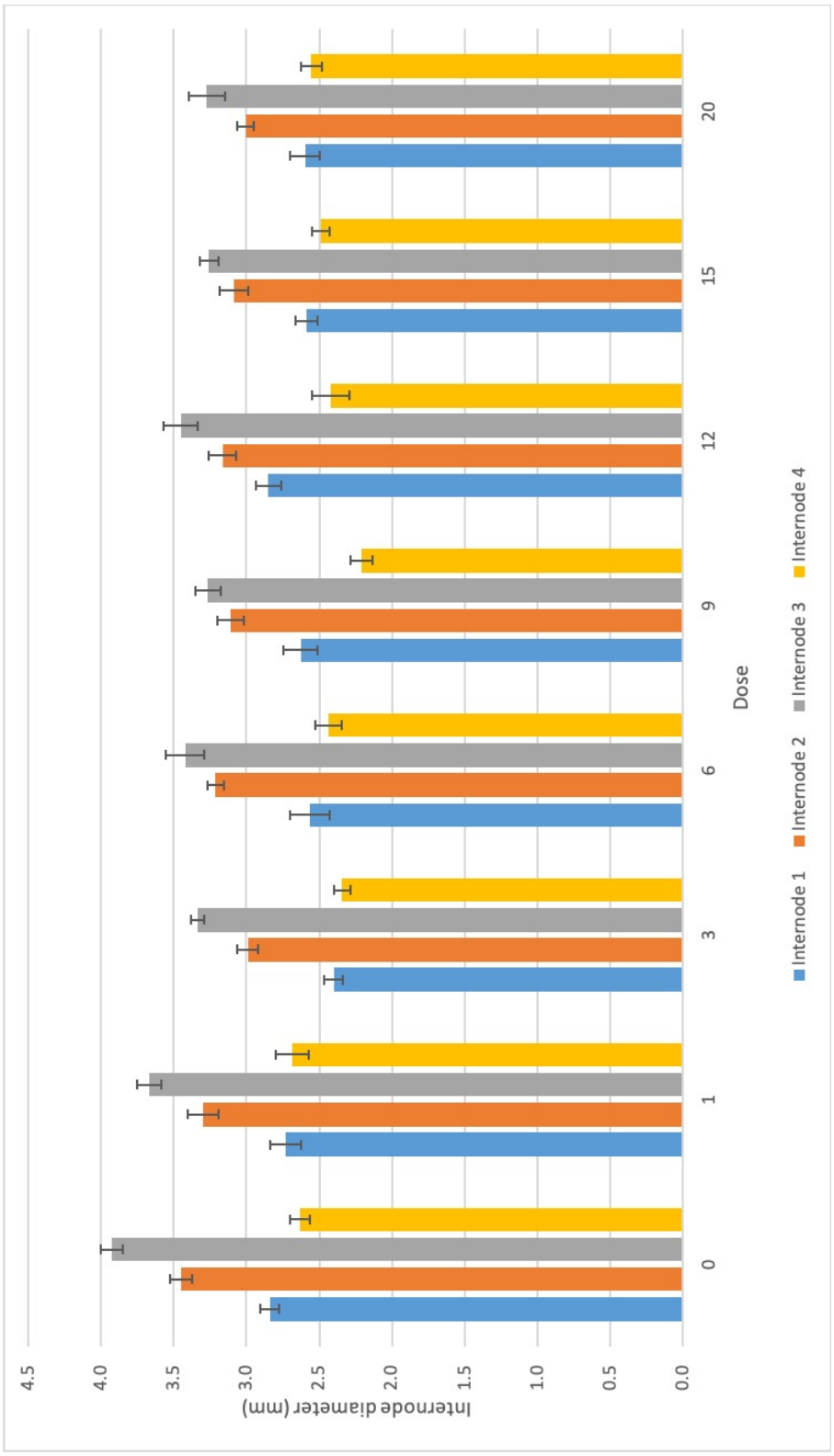


Figure 30 - Internode diameter at the end of flowering following (T2) earlier treatment of increasing dosses of brushstrokes per day Internodes numbered from bottom (1) to top (4) in response to increasing doses of brushing treatment. Error bars indicate ±1 Standard error of the mean.

4.3.5 Flag leaf measurements

Treated plants had a longer flag leaf on the main tiller than untreated plants (Figure 31). The average flag leaf length of untreated plants was 29.93cm, while 1 brushstroke resulted in an increase of 3.46cm and 20 brushstrokes a 2.20cm increase. Only 1, 6, and 12 brushstroke treatments resulted in significantly different flag leaf lengths compared with untreated plants (Dunnett t, $p<0.01$ for 1 and 6 brushstrokes, $p<0.05$ for 12 brushstrokes).

Treatments had very little effect on leaf width, with most doses resulting in slightly narrower leaves than controls. However, plants that received 6 brushstrokes had slightly wider leaves than controls (data not shown).

There was no clear trend in flag leaf area in response to increased doses of treatment (Figure 32). Some doses increased flag leaf area (1, 6, 12, and 20), while other doses decreased area (3, 9, and 15). These differences were very small and not significant.

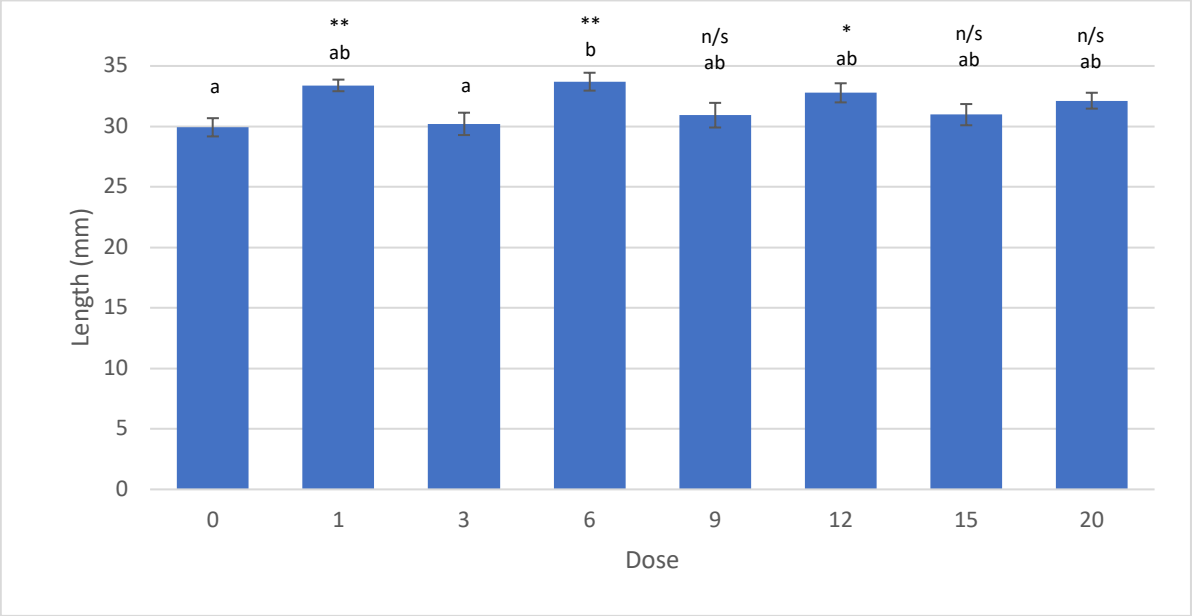


Figure 31 - Mean main tiller flag leaf length at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

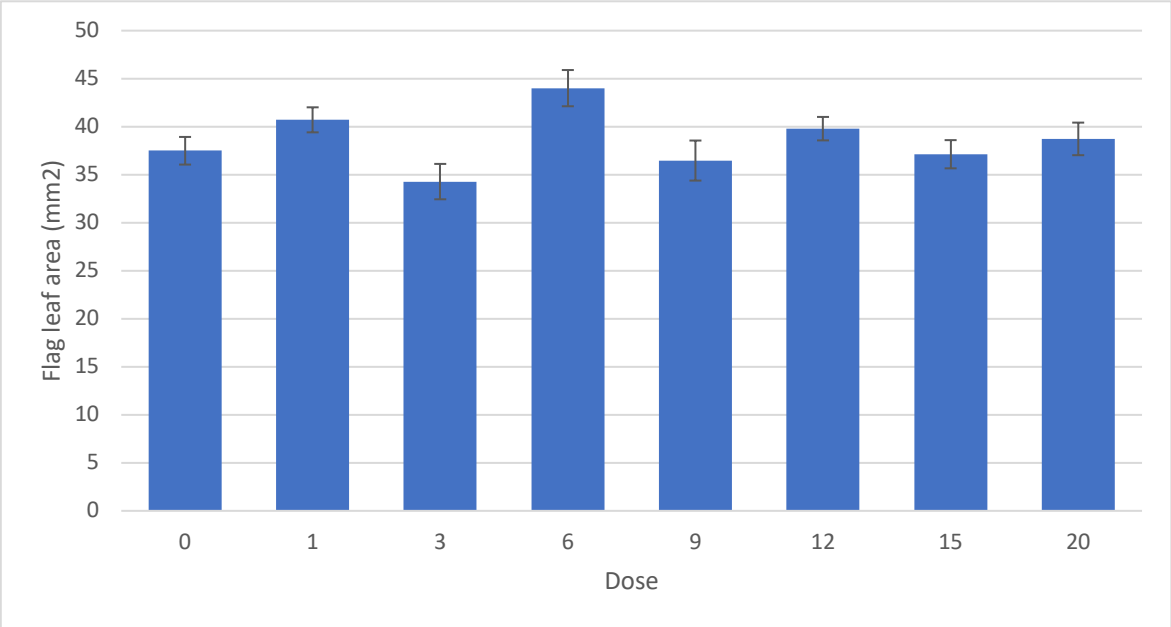


Figure 32 - Mean main tiller flag leaf area at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=20.

4.3.6 Tillers and flowers

4.3.6.1 T1 Tillers

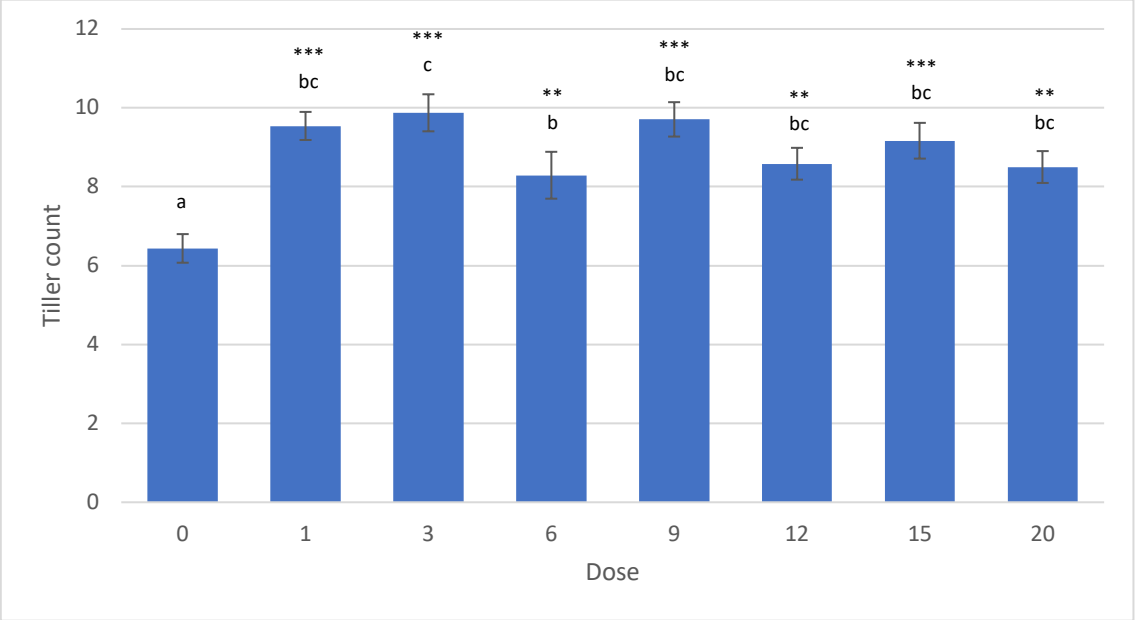


Figure 34 - Number of tillers per plant at the end of treatment (T1). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

At the end of treatment, the number of tillers on each plant was counted (Figure 34). Treated plants had significantly more tillers per plant than untreated plants (ANOVA p<0.001), though there was no consistent pattern in the number of tillers relating to the number of brushstrokes applied. Untreated plants had an average of 6.3 tillers par plant, compared with 9.5 tillers after 20 brushstrokes. 3 Brushstrokes had the greatest effect, increasing tilers by 3.9 and 6 brushstrokes was the least effective, increasing tiller numbers by only 2 on average. 1, 3, 9, and 15 brushstrokes had a highly significant effect on tiller numbers (Dunnett t p<0.001) followed by 6, 12 and 20 brushstrokes (p<0.01). There was no statistically significant difference in tiller numbers between all of the treatments.

4.3.6.2 T2 number of flowers

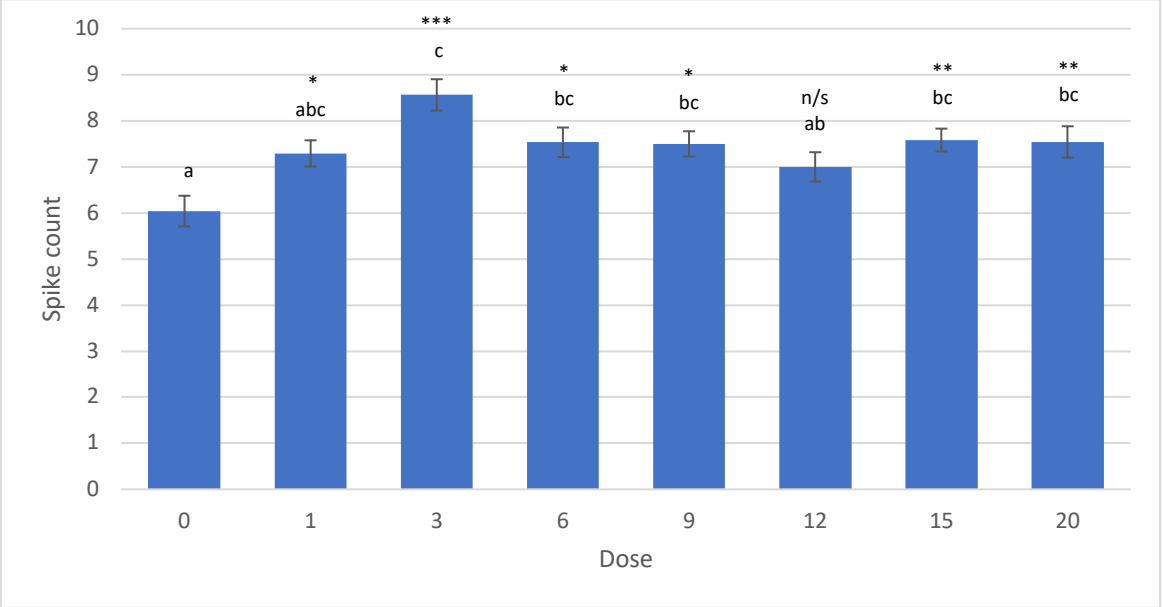


Figure 35 - Average numbers of flowering spikes per plant at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

Treatment resulted in an increase in the number of flowering spikes per plant compared with untreated plants (Figure 35). While untreated plants had an average of 6.0 flowers per plant,

3 brushstrokes had the greatest effect, with an average of 8.6 flowers, and 12 brushstrokes yielded the least difference, with an average of 7 flowers per plant. The application of 1 brushstroke per day increased flower numbers by 1.3, and plants that received 20 brushstrokes had 1.5 more flowers per plant than untreated ones on average. All treatments bar 12 brushstrokes resulted in a significant difference in flower numbers compared with untreated plants.

4.3.6.3 Main tiller spike weight

When the plant had become completely senesced, final phenotypic measurements were taken. Spikes were cut from the stems 1cm below the flower and tagged if a main tiller, or numbered according to height order, form tallest to shortest tiller on the plant. Main tiller spikes were weighed individually.

Table 3 - Weight of spikes from senesced mature plants (T3).

Treatment	0	1	3	6	9	12	15	20
n	6	6	6	6	6	6	6	6
Main tiller spike weight (g)								
Mean	1.88	1.41	1.12	1.45	1.25	0.97	1.07	0.98
Std. Deviation	0.247	0.184	0.393	0.133	0.249	0.380	0.345	0.247
SEM	0.101	0.075	0.160	0.054	0.102	0.155	0.141	0.101
Sig.		*	***	n/s	**	***	***	***
	b	ab	a	ab	a	a	a	a
Weight of all spikes per plant (g)								
Mean	11.254	10.076	11.283	11.051	9.874	9.220	10.439	9.786
Std. Deviation	2.0756	0.5501	0.7664	1.5654	1.3643	2.3516	1.4066	2.0691
SEM	0.847	0.225	0.313	0.639	0.557	0.960	0.574	0.845
Sig.		n/s	n/s	n/s	n/s	n/s	n/s	n/s
Average spike weight per plant (g)								
Mean	1.792	1.309	1.289	1.387	1.231	1.223	1.223	1.228
Std. Deviation	0.1728	0.1756	0.1249	0.1082	0.2011	0.2699	0.2332	0.1072
SEM	0.071	0.072	0.051	0.044	0.082	0.110	0.095	0.044
Sig.		***	***	**	***	***	***	***
	b	a	a	a	a	a	a	a

Brushing had a significant effect on main tiller weight (ANOVA, $p<0.001$) and resulted in a clear reduction compared with untreated plants (Table 3). 12 brushstrokes had the greatest effect, reducing average main tiller spike weight by 0.917g compared with untreated plants. Six brushstrokes did not have a significant effect on main tiller spike weight.

Compared against untreated plants 3, 12, 15, and 20 brushstrokes had a highly significant effect on main tiller spike weight (Dunnett t $p<0.001$) and was more effective than 9 brushstrokes ($p<0.01$) and 1 brushstroke ($p<0.05$).

4.3.6.4 Total spike weight per plant

All spikes from each plant were weighed together to determine total spike weight (Table 3). The general trend was a reduction in total spike weight following treatment, except for plants that received 3 brushstrokes, which were on average 0.029g more than untreated plants (11.254g per plant average). Of the treatments where, total spike weight was reduced, 12 brushstrokes was the most effective, with an average total spike weight of 9.220g, and 6 brushstrokes was the least effective with an average of 11.051g. Overall, none of the treatments were significantly effective and there was no significant difference between treated and untreated plants.

4.3.6.5 Average spike weight per plant

The weight of all spikes from each plant was divided by the number of spikes the plant yielded, to determine an average spike weight for the plant (Table 3). When looking at the average weight of spikes for each plant, there was a significant difference between treated and untreated plant (ANOVA $p<0.001$). There was a steady reduction in average spike weight as the number of brushstrokes applied increased, apart for 6 brushstrokes, which resulted in the highest average spike weight for any of the treatments (1.387g). A Dunnett t post hoc test indicated that all treatments were highly significant at $p<0.001$, except for 6 brushstrokes,

which was significant at $p<0.01$. A Tukeys test showed no significant differences in average spike weight between treatments.

4.3.7 T3 Biomass

Table 4 - Whole plant and Main tiller biomass of mature plants (T3).

Treatment	0	1	3	6	9	12	15	20
n	6	6	6	6	6	6	6	6
Whole plant biomass (g)								
Mean	6.14	6.79	6.96	7.69	6.22	6.89	6.43	6.58
Std. Deviation	1.173	0.528	0.465	1.293	0.572	1.663	0.523	0.797
SEM	0.479	0.216	0.190	0.528	0.233	0.679	0.214	0.325
Sig.		n/s	n/s	n/s	n/s	n/s	n/s	n/s
Main tiller biomass (g)								
Mean	0.586	0.444	0.364	0.464	0.422	0.360	0.347	0.344
Std. Deviation	0.0600	0.0447	0.0934	0.0479	0.0742	0.0718	0.1269	0.0607
SEM	0.0245	0.0183	0.0381	0.0196	0.0303	0.0293	0.0518	0.0248
Sig.		*	***	n/s	**	***	***	***
	b	a	a	ab	a	a	a	a

4.3.7.1 Whole plant biomass

For each plant, senesced above ground material (excluding flowering spikes) was weighed to determine whole plant biomass (Table 4). Treated plants had an increased total biomass compared with untreated. Untreated plants had an average total biomass of 6.138g while plants that received 1 brushstroke were 6.578g on average. Of the treatments, 9 brushstrokes were the least different in weight to untreated plants (0.083g) and 6 brushstrokes were the most different, weighing 1.550g more than treated plants on average. Overall, none of the treatments had a significant effect on total plant biomass.

4.3.7.2 Main tiller biomass

After the entire plant had been weighed, the main tiller was separated and weighed separately (Table 4).

Across all treatments, there was a reduction in main tiller weight. There was a general downward trend in main tiller weight as the number of brushstrokes increases, except for 6 and 9 brushstrokes treatments. Main tillers of untreated plants weighed 0.586g on average. Plants that received 1 brushstroke weighed 24% less and plants that received 20 brushstrokes weighed on average 58% less, however the main tillers of 6 brushstroke treated plants were only 20% less than controls. A Dunnett t statistical test indicated that 3, 12, 15, and 20 brushstrokes had a highly significant effect ($p<0.001$) followed by 9 brushstrokes ($p<0.01$) and 1 brushstroke ($p<0.05$) compared with untreated plants. There was no significant difference in main tiller biomass between plants that received 6 brushstrokes and those that received no treatment.

4.3.8 Stem mechanical properties

Due to the effectiveness of treatment in reducing plant height, and particularly the length of the second internode, there were too few internodes over 5 cm in length to conduct effective mechanical tests for 3, 9 and 12 brushstroke treatments. Therefore, these treatments have been excluded when comparing the effects of treatment on the second internode.

4.3.9 Mechanical properties of main tiller internodes 2 and 3 at the end of flowering (T2)

4.3.9.1 Cross-section stem diameter of the second and third internodes t T2

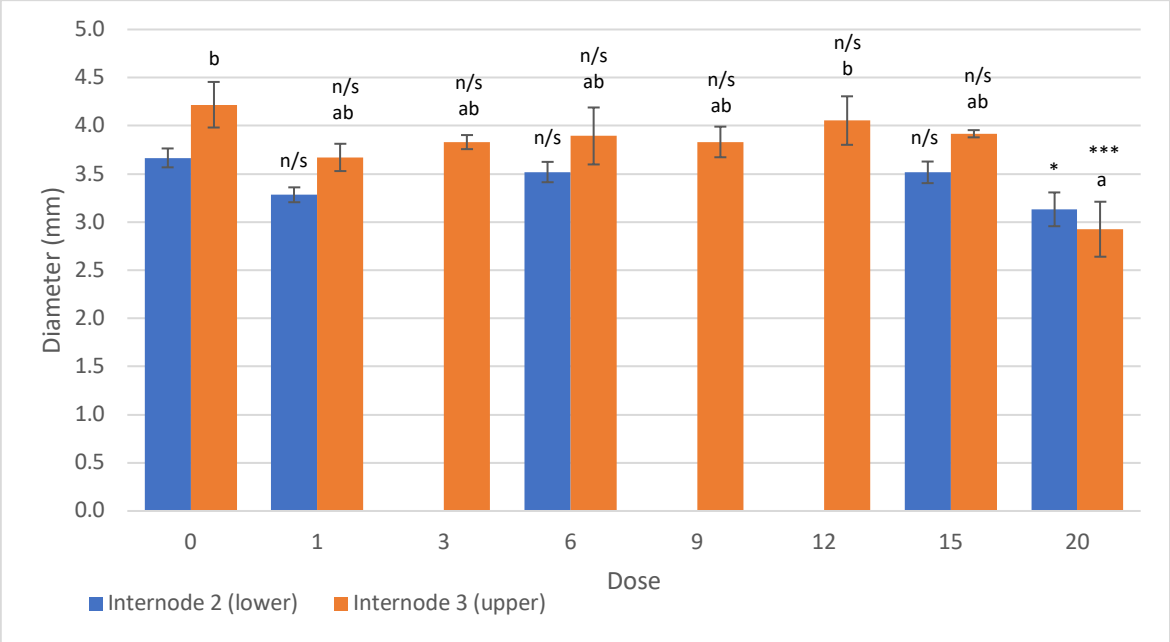


Figure 36 - Stem diameter at the end of flowering (T2). Diameter of internode 1 and 2 measured close to the site of impact from 3-point bending hammer. At T2 the second internode of some plants were too short for 3-point bending tests, therefore were excluded from mechanical measurements. Where n for a treatment was fewer than 4, data was excluded completely. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$). Internode 2 n=4, Internode 3 n=6.

Treated plants had a smaller diameter of both internode 2 and three compared with treated plants (Figure 36). Untreated plants had an average internode 2 diameter of 3.67 mm and 4.22 mm for the third internode. One brushstroke reduced the diameter of internode 2 by 0.383 mm and internode 3 by 0.547 mm on average compared with untreated plants. Plants

that received 20 brushstrokes were narrower on average by 0.534 mm for internode 2 and 1.293 mm for internode 3 compared with untreated plants. All treatments had a reduced diameter of both internode 2 and 3, but only 20 brushstrokes had a significant effect on the diameter of internode 2 (Dunnett t $p<0.05$) and internode 3 (Dunnett t $p<0.01$).

4.3.9.2 Cross section area of the second and third internodes t T2

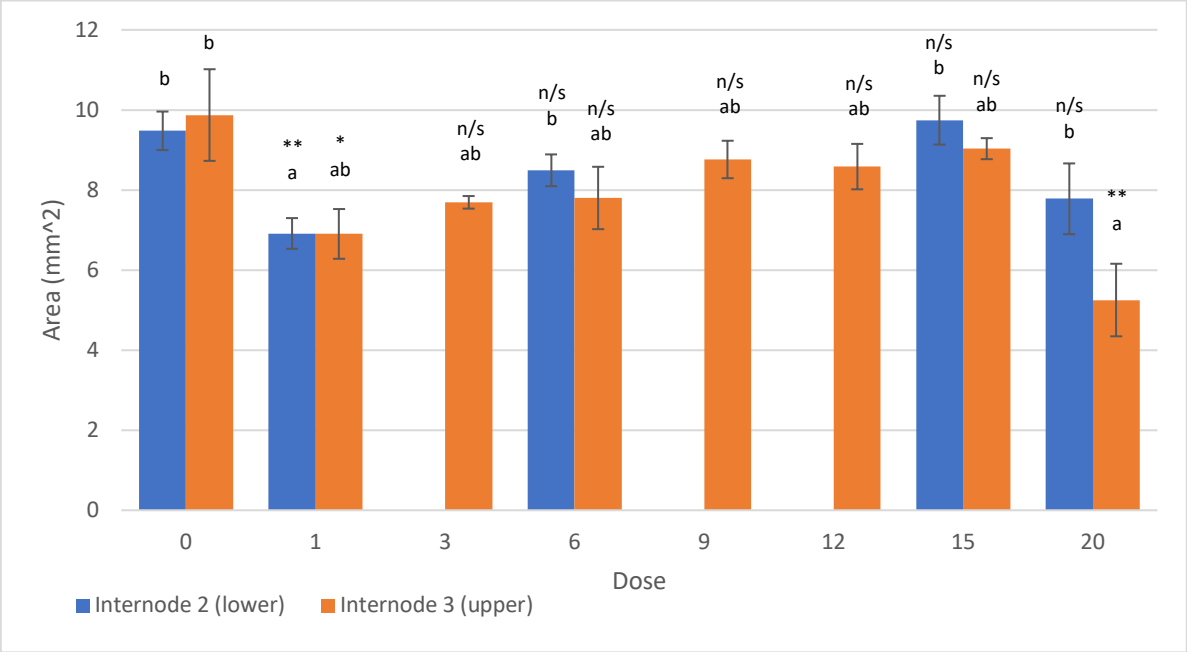


Figure 37 - Stem area at the end of flowering (T2). Area of internode 1 and 2 measured close to the site of impact from 3-point bending hammer. At T2 the second internode of some plants were too short for 3-point bending tests, therefore were excluded from mechanical measurements. Where n for a treatment was fewer than 4, data was excluded completely. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$). Internode 2 n=4, Internode 3 n=6.

Most treated plants had a lower cross-sectional area of internode 2 than untreated plants, except for those that received 15 brushstrokes, which had an average area of 9.743 mm^2 compared with 9.478 mm^2 for untreated plants (Figure 37). However, the third internode of treated plants had a reduced cross-section area across all treatments compared with untreated. Plants that received 1 brushstroke had an average internode 2 cross-sectional area of 6.914 mm^2, while 20 brushstroke plants were 7.780 mm^2 on average. Only 1 brushstroke had a significant effect on the cross-sectional area of internode 2 (Dunnett t $p<0.01$). On the

other hand, both 1 and 20 brushstrokes significantly affected the area of the third internode (Dunnett t $p<0.05$ and $p<0.01$ respectively), reducing internode 3 cross-sectional area by 2.969 mm^2 and 4.607 mm^2 respectively.

4.3.9.3 Bending strength of the second and third internodes t T2

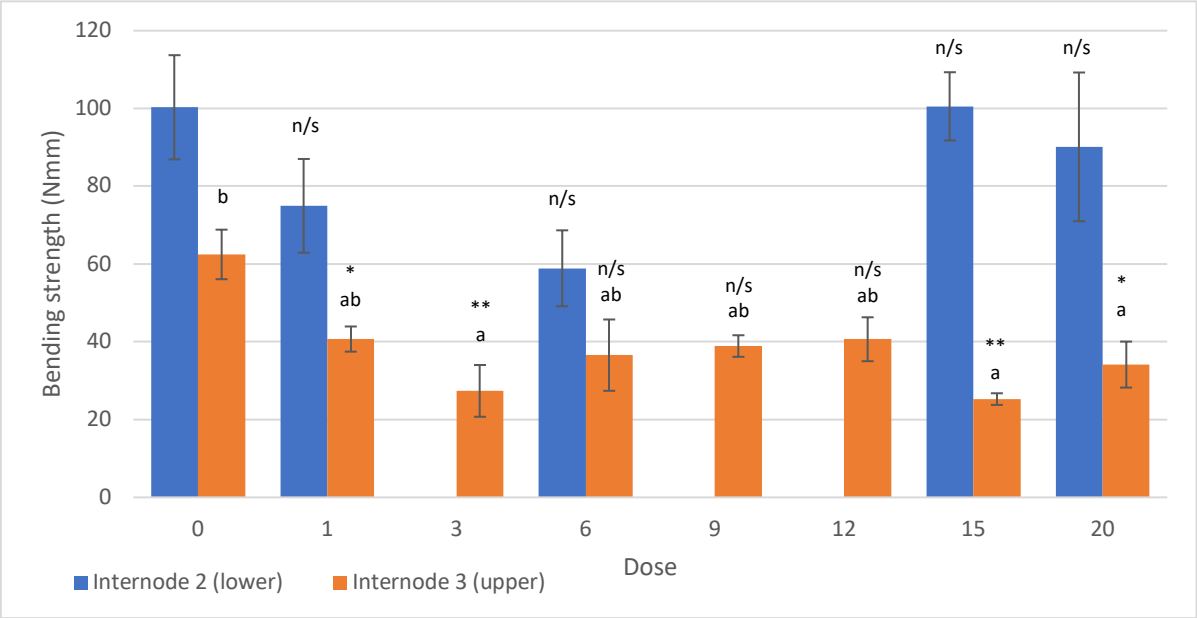


Figure 38 - Bending strength of internode 2 and 3 at the end of flowering (T2). At T2 the second internode of some plants were too short for 3-point bending tests, therefore were excluded from mechanical measurements. Where n for a treatment was fewer than 4, data was excluded completely. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$). Internode 2 n=4, Internode 3 n=6

Though the second internode of treated plants had a lower bending strength, it was not statistically significant. However, all treatments resulted in a reduced bending strength of internode 3 compared with untreated plants (Figure 38). Plants that received 1 brushstroke had an average bending strength of 40.71 Nmm, 21.759 Nmm less than untreated plants and 15 brushstrokes resulted in an average bending strength of 37.195 Nmm. Both 3 and 15 brushstrokes were highly effective treatments and statistically significant at $p<0.001$, followed by 20 brushstrokes at $p<0.01$. 1, 6, and 9 brushstrokes also had a significant effect

(p<0.05), though 12 brushstrokes did not have a significant effect on the bending strength of internode 3.

4.3.9.4 Bending rigidity of the second and third internodes t T2

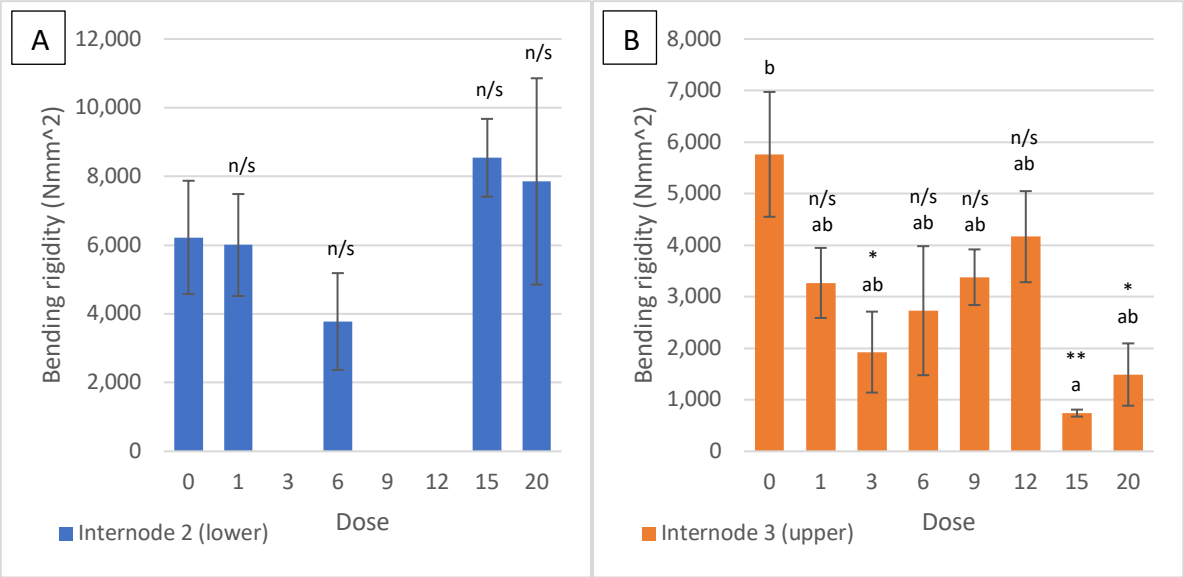


Figure 39 – Bending strength of internode 2 (A) and 3 (B) at the end of flowering (T2). At T2 the second internode of some plants were too short for 3-point bending tests, therefore were excluded from mechanical measurements. Where n for a treatment was fewer than 4, data was excluded completely. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$). Internode 2 n=4, Internode 3 n=6

Plants that received either 1 or 6 brushstrokes per day had a lower bending rigidity of the second internode, whereas 15 and 20 brushstrokes resulted in an increase in bending rigidity compared with plants that received no treatment (Figure 39a). None of the treatments had a significant effect on the bending rigidity of the second internode.

There was a clear trend in the bending rigidity of internode 3 in response to treatment - untreated plants had a higher bending rigidity than plants that received treatment (Figure 39b). Plants that received 1 brushstroke had an average bending rigidity of 3267.1 Nmm^2 and 3 brushstrokes resulted in an average of 1924.8 Nmm^2, while increasing the number of brushstrokes to 6, 9, and 12 resulted in an increase in bending rigidity. Plants that received

12 brushstrokes had an average bending rigidity of 4164.6 Nmm^2, the highest of any treatment. 15 brushstrokes were the most effective in reducing bending rigidity, with an average of 1489.4 Nmm^2, significantly lower than untreated plants ($p<0.01$). 20 brushstrokes also had a statistically significant effect on bending rigidity at $p<0.05$.

4.3.9.5 Young's modulus of the second and third internodes t T2

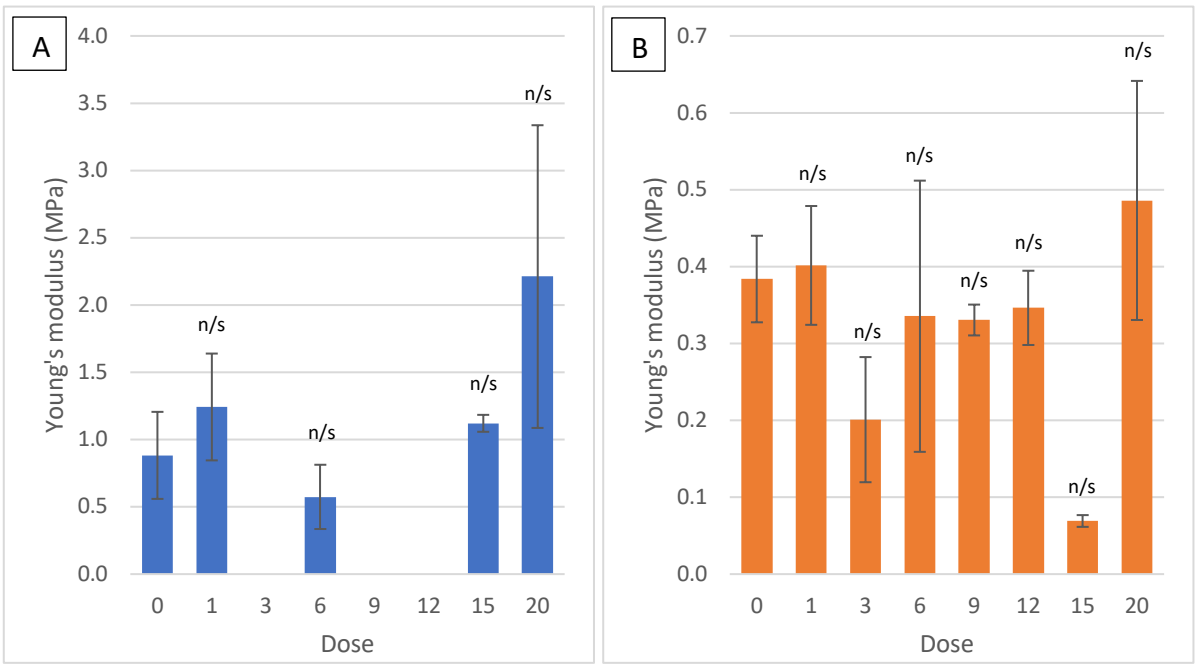


Figure 40 – Young's modulus of internode 2 (A) and 3 (B) at the end of flowering (T2). At T2 the second internode of some plants were too short for 3-point bending tests, therefore were excluded from mechanical measurements. Where n for a treatment was fewer than 4, data was excluded completely. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$). Internode 2 n=4, Internode 3 n=6

There was no overall trend in the Young's modulus of internode 2 of treated plants – 1, 15 and 20 brushstrokes resulted in an increase while 6 brushstrokes reduced the Young's modulus compared with plants that received no treatment (Figure 40a). None of the treatments had a statistically significant effect on the young's modulus.

There was an increase in young's modulus for plants that received 1 and 20 brushstrokes, but all other treatments resulted in a lower young's modulus of the third internode compared with untreated plants (Figure 40b). Untreated plants had an average Young's modulus of 0.384 GPa while 15 brushstrokes resulted in the lowest (0.069 GPa) and plants that received 20 brushstrokes had the highest young's modulus of plants that received treatment (0.486 GPa). None of the treatments had a significant effect on the young's modulus of internode 3.

4.3.10 Mechanical properties of the third internode of main tiller stems at maturity (T3)

Once plants had become fully senesced, a 50mm section was cut from the middle of the third internode of each main tiller for the measurement of stem mechanical properties. Each section was subjected to a 3-point bending test, and then sectioned close to the impact site to measure the inner and outer stem diameter. Cross-section measurements were then used with data from the 3-point bending test to calculate stem cross-sectional area, stem wall thickness, bending strength, bending rigidity and Young's modulus.

4.3.11 Stem diameter at T3

4.3.11.1 Inner diameter of the third internode at T3

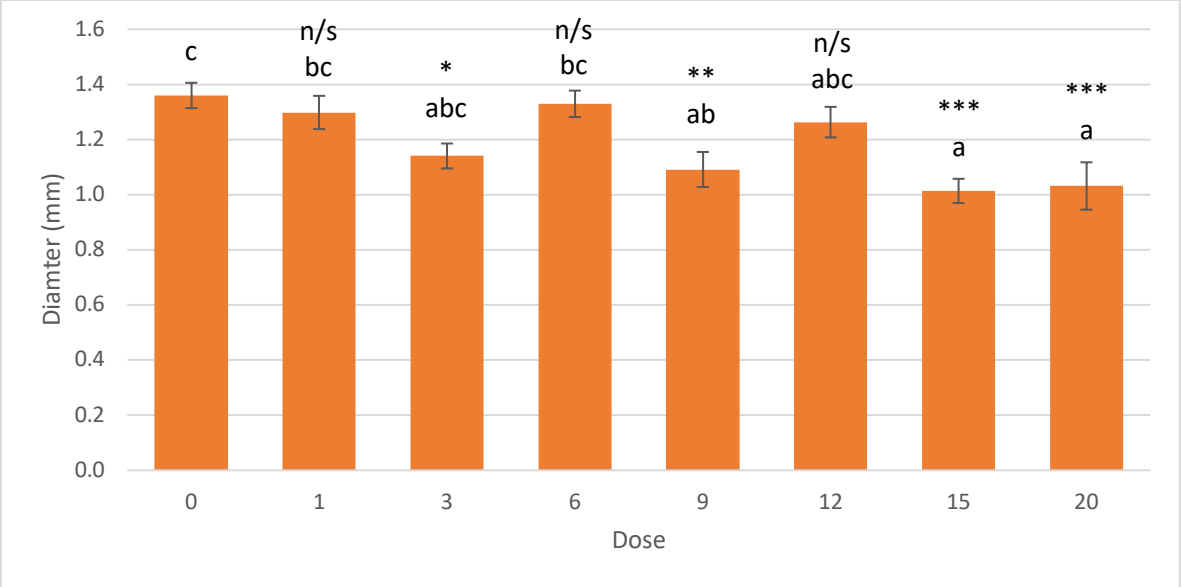


Figure 41 - Inner diameter of the third internode (T3). Measurements taken from images of cross-sections obtained from stem sections used in 3-point bending tests. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=6.

Treated plants had a significantly smaller inner diameter of internode 3 than untreated plants (ANOVA, $p<0.001$). Plants that received 1 brushstroke were on average 0.06 mm smaller than untreated, though not significantly different (Figure 41). 6 and 12 brushstrokes also did not

have a significant effect on the inner diameter of internode 3. In comparison, both 15 and 20 brushstrokes were highly effective ($p<0.001$), with an average inner diameter of 1.01 mm and 103 mm respectively, followed by 9 brushstrokes ($p<0.01$) and 3 brushstrokes ($p<0.05$).

4.3.11.2 Outer diameter of the third internode at T3

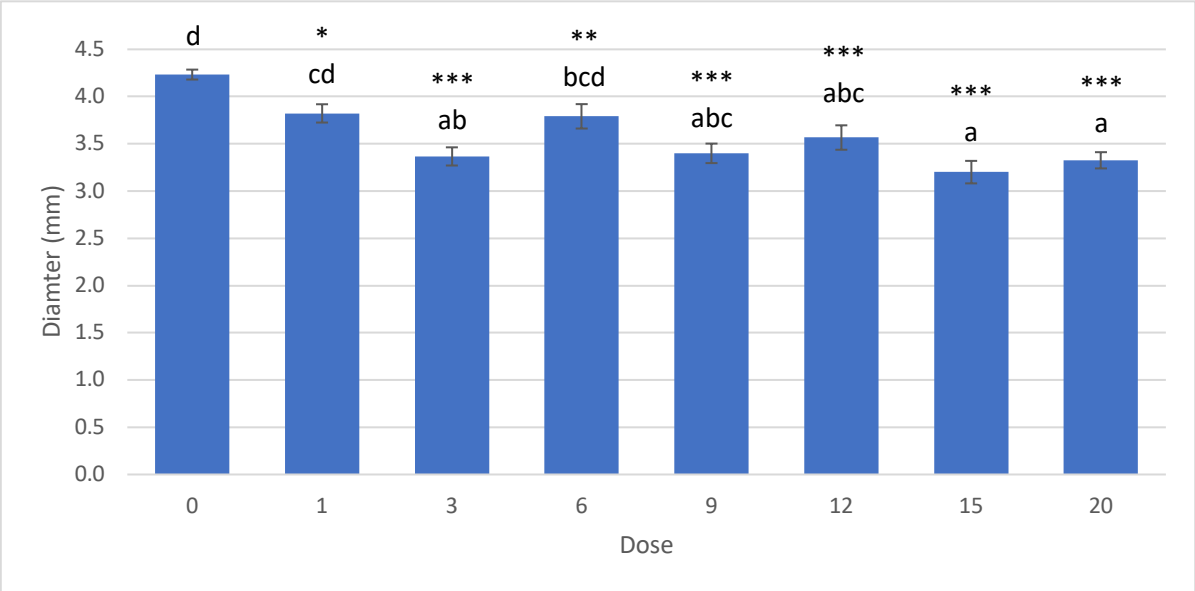


Figure 42 - Outer diameter of the third internode (T3). Measurements taken from images of cross-sections obtained from stem sections used in 3-point bending tests. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

Treatment significantly reduced the outer diameter of the third internode (ANOVA $p<0.001$), with untreated plants having an average outer diameter of 4.233 mm and treated plants between 3.822 mm (1 brushstroke) and 3.201mm (15 brushstrokes). The effect of 3, 9, 12, 15 and 20 brushstrokes were highly significant (Dunnett t $p<0.001$) compared against untreated plants (Figure 42).

4.3.11.3 Cross sectional area of the third internode at T3

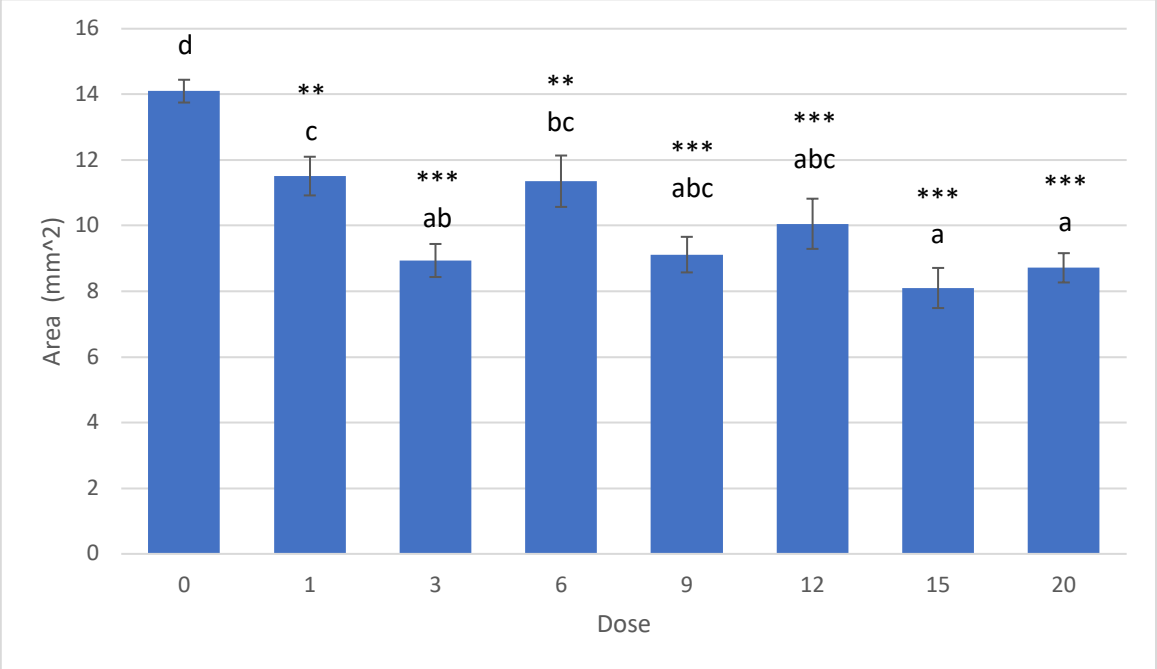


Figure 43 - Cross section area of the third internode of senesced mature stems (T3). Measurements taken from images of cross-section near impact site of 3-point hammer. Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6

Treated plants had a significantly smaller cross-section area of internode 3 (ANOVA p<0.01) with a trend in the response to treatment closely related to that of the outer diameter measurements (Figure 43). Again, applying 9 or more brushstrokes once per day had a highly significant effect on the cross-sectional area of the third internode (p<0.001).

4.3.11.4 Bending strength of the third internode at T3

Treatment of plants resulted in a significantly reduced bending strength (ANOVA p<0.001). As the number of brushstrokes increased, stem bending strength generally reduced, from 27.22 Nmm for untreated plants to 19.89 Nmm after 1 brushstroke daily, to 14.48 Nmm after 20 daily brushstrokes (Figure 44). 3, 9, 12, 15, and 20 brushstrokes were significant at p<0.001, and 1 and 6 brushstrokes at p<0.01.

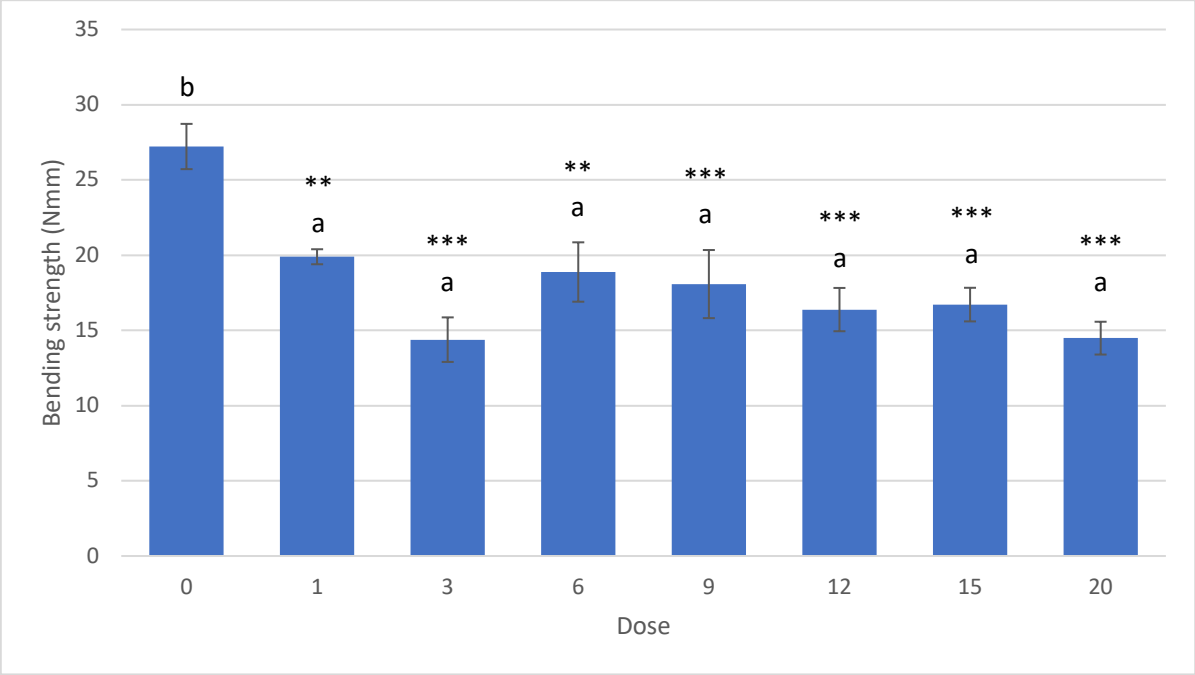


Figure 44 - Bending strength of the third internode from main tillers of senesced mature plants (T3). Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

4.3.11.5 Bending rigidity of the third internode at T3

Treated plants also had a significantly lower bending rigidity (ANOVA p<0.001), and a very similar trend to the bending strength (Figure 45). Bending rigidity reduced with increasing number of brushstrokes, apart from 3 brushstrokes, which resulted in a much lower bending rigidity – 815.8 Nmm^2 compared with 117.1 Nmm^2 after just 1 brushstroke and 685.4 Nmm^2 after 20.

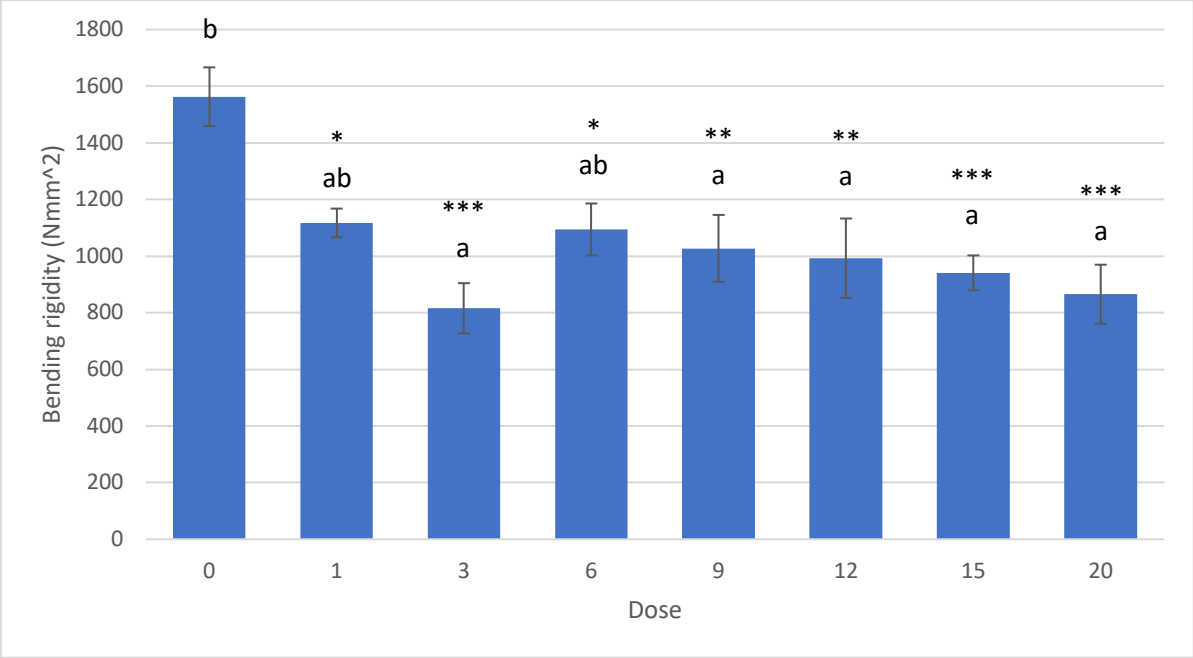


Figure 45 - Bending rigidity of the third internode from main tillers of senesced mature plants (T3). Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

4.3.11.6 Young's modulus of the third internode at T3

The third internode of treated plants had a greater Young's modulus than untreated plants, with more brushstrokes generally resulting in a greater effect (Figure 46). Untreated plants had an average young's modulus of 0.122 GPa while plants that received 1 brushstroke had a young's modulus of 0.142 GPa and 0.234 GPa after 15 brushstrokes. Only 15 brushstrokes had a significant effect on the young's modulus of the third internode of main tillers (Dunnett t $p<0.01$).

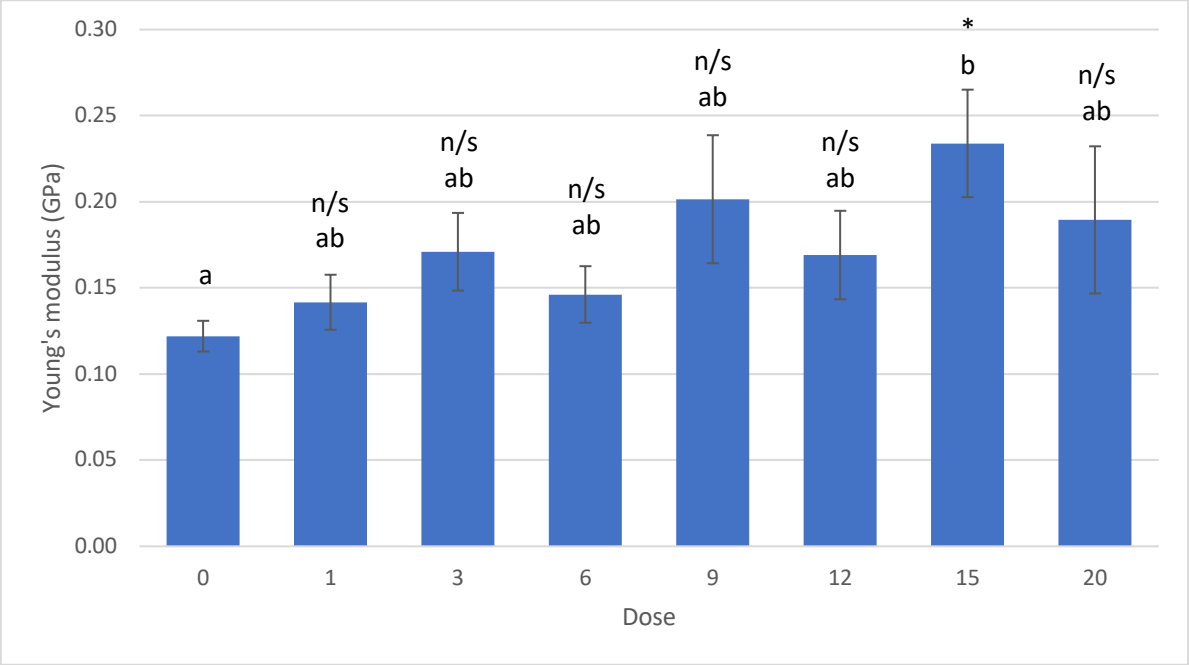


Figure 46 - Young's modulus of the third internode from main tillers of senesced mature plants (T3). Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

4.3.12 Grain measurements and yield

Grain measurements were obtained through analysis of the images produced by micro CT scanning. Initial volume measurements consisted of the total connected pixels in each grain, which was converted into mm^3. Data was screened to remove the top and bottom 5% due to a large amount of non-grain materiel picked up by the image analysis software. The volume of each individual grain in the spike was measured and totalled to give a total grain volume for each spike. A count of grains was then derived from the number of volume measurements per spike.

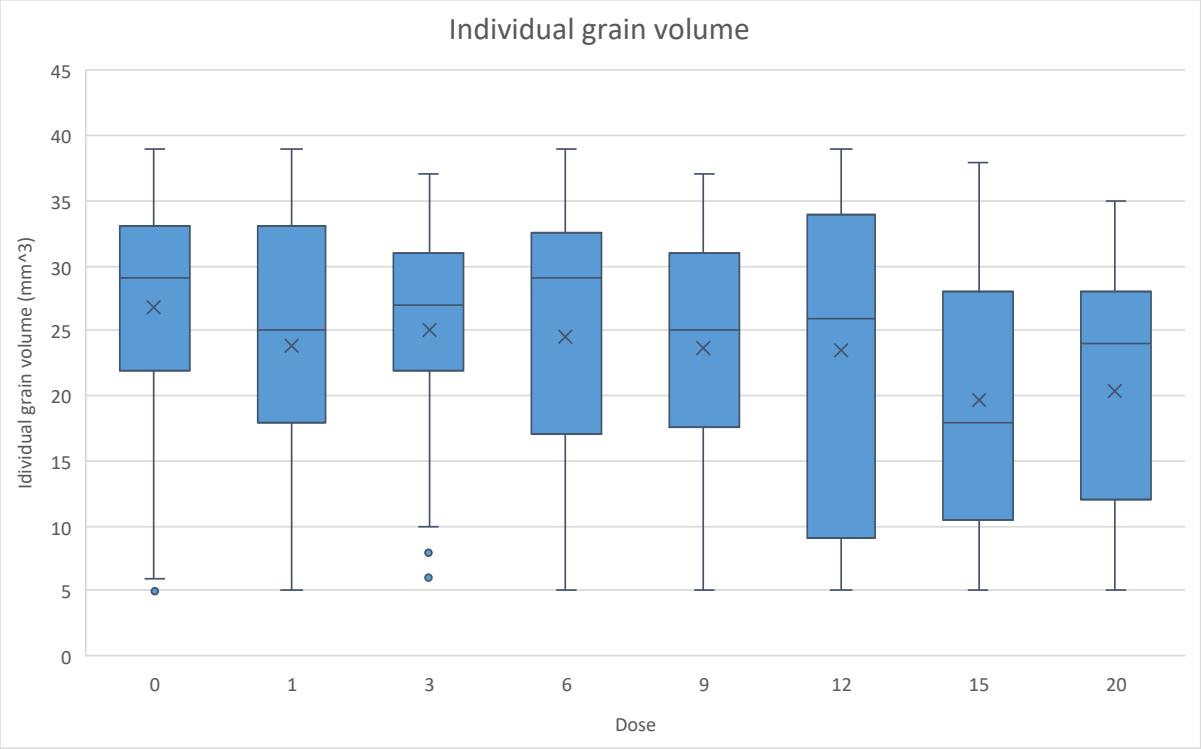


Figure 47 - Individual grain volume of mature plants (T3) following earlier treatment. In this chart, the upper whisker indicates the top 25% of data, excluding outliers, and the lower whisker indicates the lowest 25% of data, excluding outliers which are marked as dots beyond the whiskers. The X indicates the sample mean and the median value is indicated by the line inside the box. The box itself indicates the interquartile range, where 75% of measurements fall.

Treatment resulted in a reduction in average individual grain volume, with a general decline in grain volume as the dose of brushing treatment increased (Figure 47). The average

individual grain volume of plants receiving 3 brushstrokes was 25.02 mm^3, while the grains of plants receiving 15 brushstrokes measured 219.38 mm^3. Only 15 and 20 brushstrokes had a significant effect on average individual grain volume compared with controls, $p<0.001$ (Dunnett t).

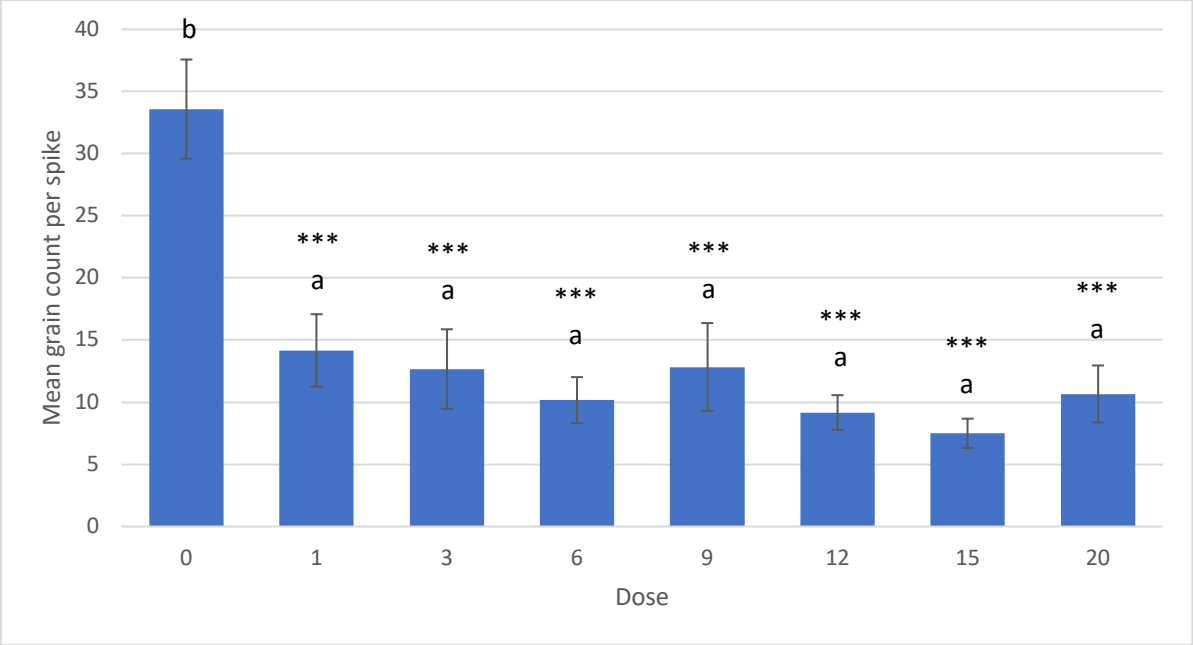


Figure 48 - Number of grains per spike at maturity (T3). Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

All treated plants had significantly fewer grains per spike than untreated plants, $p<0.001$ Dunnett t (Figure 48). Grain number generally decreased in line with increasing treatment dose, however there was no significant difference between treatments. Plants that received treatment had between 14.2 grains (1 brushstroke), and 7.5 grains per spike (15 brushstrokes), compared with an average of 33.6 grains per spike for controls.

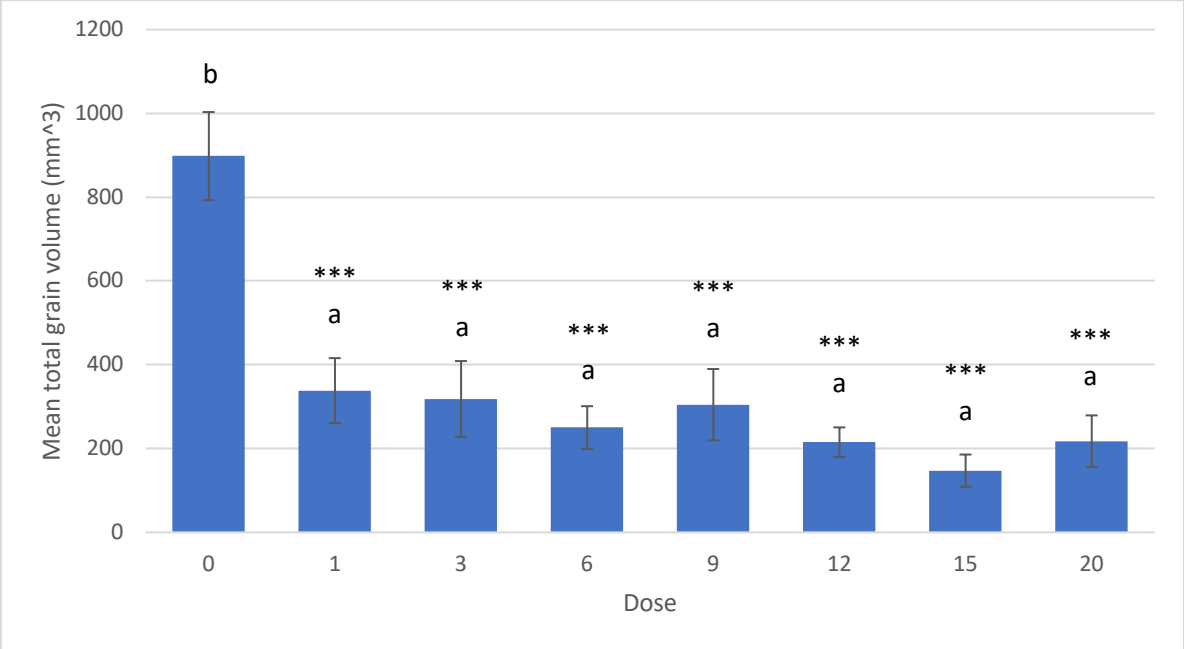


Figure 49 - Total volume of all grains in each spike at maturity (T3). Error bars indicate ±1 SEM. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test ($\propto = 0.05$) n=6.

Treated plants had significantly lower total grain volume per spike than untreated plants, $p<0.001$ (Dunnett t). While untreated plants had an average spike total grain volume of 898.0 mm^3, plants that received 1 brushstroke had an average total volume of 338.5 mm^3 and 15 brushstrokes 147.2 mm^3 (Figure 49). There was no significant difference between treatments.

4.4 Discussion

4.4.1 Plant height

A significant reduction in the height of treated plants was observed when data was collected at the end of treatment, and difference in height between treated and untreated increased with increasing doses of treatment. All treatments resulted in a significant reduction in height compared with untreated plants (controls). However, when the height of main tillers was recorded at the end of flowering, the response was much less consistent, with only 3, 12, 15, and 20 brushstrokes having a significant effect. Further, when average plant height was measured, there was no significant effect across all treatments.

The trend in the height of plants seen at the end of treatment in response to increasing dose is in agreement with results seen in other studies. Both Telewski and Pruyn (1998) and Autio et al. (1994) found a linear trend in height response to increasing levels of brushing treatment. Garner and Bjorkman (1996), found a reduction in the height of tomato plants in response to brushing, and increasing dose generally increased the response, though the trend was not linear.

Plants that received the largest doses of treatment had the largest difference in height to controls, and this trend continued through to flowering and beyond. In contrast, plants that received lower doses of treatment grew to a height similar to control plants by the end of flowering. The reduction in difference between treated and untreated plants between early and later measurements indicates that growth recovers after the cessation of treatment. Only

3, 12, 15 and 20 doses of treatment had a lasting effect on plant height, when measured at the end of flowering, with 3 brushstrokes having the most significant lasting effect.

It is interesting to note that even one brushstroke, the lowest treatment in this experiment, reduced height compared with controls. At the end of treatment, the effect was significant, while data collected after flowering indicated that though treated plants were still smaller than controls, the difference was no longer significant.

Garner and Bjorkman (1996) suggest that responses to very low levels of mechanical stimulation, such as from handling plants, watering or greenhouse ventilation, may not be enough to have an effect on plant height. They suggest that these low levels of mechanical stimulation may be very easily saturated and may provide more of a developmental cue than a stress induced reduction in growth. Therefore, the lowest level of treatment in the present experiment, one brushstroke, must be providing enough mechanical stimulation to be over the minimum threshold for an effect on growth in these wheat plants. In contrast, plants such as Aster, Dusty Miller, and Petunia, have a much higher threshold (60 brushstrokes or more) in order for plants to have a significant growth response (Autio, Voipio, and Koivunen 1994).

At the opposite end of the treatment range, 20 brushstrokes significantly reduced plant height during treatment. Treatment at this intensity also had a lasting effect, with plants maintaining a significant reduction in height when measured after flowering. While the threshold for an immediate response to treatment is obviously very low for wheat, there is a higher threshold for a lasting response to treatment – around 12 brushstrokes.

4.4.2 Flag leaf

The flag leaf provides the tillers main photosynthetic area and any reductions in its size, through damage or responses to stress, could reduce the plants photosynthetic capacity. Flag leaf length and width was measured after plants had finished flowering, and these measurements were used to calculate an estimated flag leaf area.

All treatments increased leaf length, though the effect on leaf area was not consistent. Only 1, 6 and 9 brushstroke treatments resulted in a significant effect on flag leaf length, while none of the treatments had a significant effect on flag leaf area. It is not clear why these treatments in particular had a greater effect on leaf length than others. Under the conditions plants for this experiment were grown in (static air in greenhouse), it is unlikely that leaves would have been excessively damaged through repeated collisions. Wind causes plants to move dynamically, which results in collisions between neighbouring plants. Leaves that come in contact with each other, and other plant parts, may suffer damage through taring or abrasion (Grace 1977, deLangre 2008). Damage to the flag leaf could affect grain filling and subsequently yield (Cleugh, Miller, and Böhm 1998). The brushing rig used in this experiment resulted in minor damage to some leaves, through folding leaves as the bar passed over. These leaves appear to not have been severely affected by the damage.

Most research on the effect of mechanical treatment has focused on damage caused to cereal plants and forage grasses through the direct effects of wind such as sand blasting, abrasion, taring, etc (see Cleugh et al., (1998), for a comprehensive review). There appears to be no references to flag leaf measurements following mechanical treatment of wheat plants. This makes drawing any specific conclusions tricky.

4.4.3 Tillers and spikes

Treated plants had significantly more tillers than controls, though there was little difference in tiller numbers between treated plants. When flowering spikes were counted, the trend continued with almost all treatments resulting in more flowering spikes per plant. However, 6 brushstrokes did not differ significantly from controls. Again, there was no significant difference in the number of spikes between treatments.

When brushed, *Lolium perenne* also had no significant change in tiller numbers (Wang et al. 2010). However, Zhao et al. (2018) found a significant increase in rice tiller numbers in response to rubbing. This increase was most significant for lower doses of treatment – 30 and 60 times per day, while treating the plants 90 times per day resulted in little difference to controls.

Tiller numbers of cereals and forage grasses may change due to environmental and management factors. Salt stress results in a reduction in tiller numbers (Goudarzi and Pakniyat 2008), as does intense drought stress, while mild drought stress actually leads to an increase in tiller numbers (Blum et al. 1990). Management practices such as altering seed density when sowing and application of growth regulators also affects tillering (Peltonen and Peltonen-Sainio 1997).

Generally, an increase in tillers at early stages of plant development results in a greater number of flowering spikes. This experiment found a significant increase in the number of flowering spikes of treated plants, though increasing treatment dose had little effect on final

spike numbers. As there was no significant difference in spike numbers between plants treated with increasing doses of brushing, the lasting effect of treatment on spike numbers seems to be the same regardless of dose.

Main tiller spikes of all but 6 dose treated plants were significantly lighter than controls. Increasing the dose of treatment resulted in decreases in main tiller weight, though the difference between treatments was not significant. There was a significant reduction in average spike weight for all tillers on each plant, though no difference between treatments. Since spike weight is strongly correlated with total volume of grains per spike (Hughes et al. 2017), it can provide an estimate of grain yield. This can be confirmed using μCT scanning, the results of which will be discussed later. There is very little information about the mass of spikes or reproductive part of cereals or forage grasses to mechanical stimulation, which makes it hard to draw a conclusion. Goodman and Ennos (1997) noted a decrease in the fresh and dry weight of the reproductive parts of maize plants that moved in the wind compared with staked plants.

4.4.4 Stem mechanical properties

At the end of flowering, the main tillers of eight plants per treatment were selected for measurements of stem mechanical properties. Fresh sections from the centre of the second and third internode were subjected to three-point bending to measure the force required to displace (bend) the stem. Stem cross-sections were obtained and measured to determine inner and outer stem diameter and calculate cross-section area. Three-point bending data and cross-section measurements were used to calculate mechanical properties of the stem, including, bending strength, bending rigidity, and Young's modulus.

Many of the treated plants had a second internode that was too short for three-point bending tests, therefore treatments that yielded too few suitable second internodes were excluded from analysis. These treatments were 3, 9, and 12 brushstrokes.

The second internode was thinner than the third, though the cross-section area was similar. 20 brushstrokes resulted in a significant reduction in the diameter of both internode two and three, and therefore also significantly reduced the cross-section area of both internodes.

The second internode consistently had a higher bending strength than the third. Brushing treatments had no effect on the bending strength of the second internode but resulted in a significant reduction in the third. Differences in bending strength between the second and third internodes were noted by Crook and Ennos (1996) in wheat and by Lemloh et al. (2014) in Sorghum. Lemloh et al. (2014) noted an increase in bending strength of the second internode between 20.1% and 98.7% and an increase of 12.6 - 71.7% following bending treatment. Goodman and Ennos (1997) noticed a 29.4% increase in the bending strength of maize plants in response to mechanical treatment compared with supported plants. However, for wheat, sorghum and maize, the increase in bending strength was associated with an increase in stem diameter. Conversely, Goodman and Ennos (1996) found no significant difference in bending strength between flexed and untreated Maize plants.

When mechanical tests were repeated using sections of the third internode of senesced mature plants, a significant reduction in bending strength was seen, which reduced with

increasing dose of brushing. Again, this was coupled with a reduction in stem diameter and cross-section area of treated plants.

The bending rigidity of fresh stems was lower for internode three of treated stems than internode two, though the bending rigidity of both internodes was similar for control plants. The third internode of both fresh and senesced samples had a lower bending rigidity following mechanical treatment, and the decline was roughly linear with dose of treatment. Crook and Ennos (1996) reported an increase in the bending rigidity of plants that received mechanical treatment. It is likely that reductions in bending rigidity are also related to reductions in stem diameter and cross-section area.

The second internode of fresh stems had a higher Young's modulus than the third, though treatment did not have a significant effect on either. The Young's modulus of the third internode of sensed stems increased roughly in line with increasing dose of treatment, though only after 15 brushstrokes was there a significant effect. Most studies reported no significant difference in Young's modulus following treatment, though Crook and Ennos (1996) did report an increase in the Young's modulus of free-standing wheat compared with supported plants.

Results from these mechanical tests indicate that both bending strength and bending rigidity are reduced in response to brushing. The internode sections measured were also thinner, with a lower cross-section area following treatment.

Calculations of the young's modulus resulted in high variability and high standard deviations as well as a lack of correlation between the Young's modulus and bending strength, indicating that the bending tests may not be accurate. This could be due to a limited resolution of strain measurement of the three-point bending machine (Lemloh et al. 2014).

4.4.5 Biomass

Despite significant increases in tiller numbers, and thus in flowering spike numbers, there was no difference in the total biomass of senesced treated plants. However, there was a significant reduction in main tiller biomass. Reductions in the biomass of main tiller stems of treated plants is likely to be related to both the reduction in height and reduction in stem diameter.

Zhao et al. (2018) noted that high doses of rubbing treatment resulted in a significant reduction in above ground biomass. Goodman and Ennos (1996) found a significant reduction in the dry weight of the stems of maize plants following flexing. However, allowing maize plants to flex freely in the wind had no significant impact on stem dry weight compared with staked plants (Goodman and Ennos 1997). Crook and Ennos (1996) also found there was no difference in stem weight between free-standing and supported wheat plants.

4.4.6 Grain yield

Plants that received treatment had significantly smaller grains in main tiller spikes than untreated plants. Treated plants also had significantly fewer grains, which resulted in a significant reduction in the total grain volume of the main tiller spikes of treated plants. There

was a noticeable increase in difference between treated and untreated plants as the dose increased.

A decrease in both grain volume and size indicates that treated wheat plants reduced assimilate partitioning to the grain during grain development or filling. This could be due to either diverting assimilates elsewhere (away from the grain), or a reduction in photosynthetic production, resulting in fewer resources during grain filling. However, while grain number was reduced by 58-78% following treatment, spike weight was reduced by only 23-48%. There is a considerable difference in the reduction in grain number compared with spike weight which cannot be accounted for. Closer investigation of weight and size of each individual part of the spike may reveal where the difference lies.

Studies on cereals and forage grasses and the effects of mechanical stress has mostly focused on growth responses of young plants, and to some extent the mechanical properties of stems. Consequently, there are no studies dealing the effect of mechanical stress on the grain or seed yield of cereals. Jaffe and Forbes (1993) reported that some shaking treatment resulted in reductions in fruit and seed production in various horticultural plants. Removing wind (and thus mechanical treatment) from plants in the field by using wind breaks had a significant effect on grain yield. Many plants, including wheat, saw an increase in the yield of sheltered crops (Grace 1977).

The reasons for reductions in yield associated with mechanical treatment are presently unclear, but Biddington (1986) suggests that the increases in respiration seen in some plants in response to mechanical stimuli might reduce net assimilation, thus affecting yield. While

mechanical treatment may be affecting growth, Biddington suggests that reductions in yield are unlikely to be a consequence of reductions in growth caused by mechanically induced stress. Jaffe and Forbes (1993) suggest that reductions in yield may actually be an advantage. Adaptations to stresses would better allow the plant to survive and reproduce, despite a reduction in yield.

Grain yield is probably the most important agronomic trait of wheat, since this is the most valuable part of the crop. Stressing plants, particularly during the grain filling stage can have severe impacts on yield. Sub-optimal photosynthesis during early grain development reduces potential grain weight (AHDB 2018). Stresses such as drought and disease can affect assimilate partitioning from anthesis through to the later stages of grain filling.

The development of wheat varieties that maintain good grain yield even during periods of stress is a key focus of breeders, and this becomes especially important when faced with a changing environment. Grain number per unit area is the common measure of yield, especially for the development of new varieties, but Foulkes et al. (2010) suggests that there needs to be more of a focus on the development of plants with larger grains.

Results from this experiment show that wheat plants produce smaller grains after periods of mechanical treatment, and it would be valuable to understand how and why this occurs. There may be trade-offs due to the plant's response to mechanical treatment. By increasing stem stiffness, fewer resources may be allocated to grain development. Foulkes et al. (2010) suggests that there may be significant dry matter costs of increasing stem strength. Since

grain filling may overlap with the end of stem expansion growth stages this could provide a reason for the reduction in yield seen in response to mechanical treatment.

This experiment found that wheat plants are highly sensitive to brushing treatment, with just one brushstroke per day having a significant effect on height (at the end of treatment), numbers of tillers and flowers and main tiller grain count. For many of the traits measured, the effect of treatment increased with increasing dose. This was clearly seen in the height difference between plants at the end of treatment, though a lasting effect on height was generally achieved by the highest doses of treatment. Where tiller numbers were found to be increased at T2, there was a lasting effect, resulting in an increase in the number of flowering spikes at T2. An increase in bending strength and rigidity of the third internode was seen following higher doses of treatment at T2, but at T3, all treatments had a significant effect on these measurements. At maturity, treated plants had fewer and smaller grains than untreated plants, and this effect increased with the size dose of treatment applied.

The next step is to look at the effect of delaying brushing, to see if sensitivity to the stimulus is affected by plant age. It will be particularly interesting to see what the effect of delaying treatment might have on the yield and grain size.

Chapter 5

5 An analysis of the response of plants of different ages to brushing treatment (Age-response)

5.1 Introduction

Biddington (1986) proposed that young plants and tissues are particularly sensitive to mechanical treatment. The response of emerging (young) tissues have been compared against older tissues following mechanical treatment. Turgeon and Webb (1971) found that petiole extension inhibition in squash was greatest for recently emerged leaves than old leaves following rubbing treatment. Brushing reduced cauliflower fresh weight most significantly in the youngest leaves (Biddington and Dearman 1985). The effect of mechanical stress on whole plants has focused more on applying the stress to plants at the same growth stage, and there have been no studies on the effect of plants of different growth stages side-by-side. A study comparing free-standing and supported wheat plants found no difference in weight but changes in mechanical properties when treatment began at the start of stem elongation (Crook and Ennos 1996). Of two varieties of wheat seedlings that had been shaken twice a day for 2.5 days, only one variety responded with a significant reduction in growth.

The other variety showed no significant response to treatment (Steucek and Gordon 1975). Chehab, Eich and Braam (2009) suggest that the reason behind these results is that young tissues are particularly sensitive to mechanical stress, and therefore must respond rapidly and drastically to survive unfavourable conditions. It is likely that as plants grow, the youngest tissues will respond strongest to treatment, therefore during each growth stage of wheat the developing tissues will be most affected. For example, during tillering, tiller initiation and growth is most likely to be affected, and during stem extension growth stages, elongation is likely to be affected. Plant stress resistance involves mechanisms such as phenological and developmental plasticity, which are caused by a reprogramming of cell differentiation following stress exposure (Chinnusamy and Zhu 2009). Phenotypic plasticity allows the plant to tweak growth patterns and avoid exposure to stress during critical phases of growth. Effective use of resources can also be achieved by adjusting growth and development during stress.

While research on the response of wheat plants to mechanical treatment is scant, much has been done to uncover the responses of wheat to drought and temperature stress applied at different stages of development. Mild drought stress applied during Tillering had little effect on yield as tillering increased following removal of the stress. Under high drought intensity, Tillering was only mildly affected, but grain numbers were reduced (Blum et al. 1990). According to Campbell et al. (1981), grain yield was most affected when moisture stress was applied during booting. At this stage, cells were undergoing meiosis to form gametes, a critical stage for establishing grain yield and highly sensitive to stress. Gupta et al. (2001) also found that applying stress at this stage resulted in significant reductions in tiller numbers and height. Gupta et al. also found that reductions in shoot dry weight, grain number and grain yield were

greater when stress was applied at anthesis than during booting. If the stress was applied prior to booting, yield was less affected, even if the stress lasted up to and beyond seed development (Campbell, Davidson, and Winkleman 1981).

Wollenweber et al. (2003) studied the effect of high temperature stress on wheat. When applied at the end of tillering/start of stem extension there was no significant on biomass but stressing plants at anthesis reduced it by 40%. Stress at anthesis also reduced grain number and weight. Gibson and Paulsen (1999) found that applying high temperature stress during early reproductive growth reduced the number of grains, whereas applying the stress during grain filling reduced grain weight. It is likely that mechanically stressing wheat plants at these stages of development would follow the same patterns of response as drought and temperature stress.

Without destructively harvesting wheat, it is hard to determine exact growth stages. Therefore, the number of days after germination can be used as a proxy for development. Results from the previous experiment showed that 20 brushstrokes resulted in a consistent and effective response. Going forward, 20 brushstrokes will be the treatment dose applied to plants in this experiment.

As can be seen, there is a lack of research on the effects of mechanical stress when applies at different stages of wheat development. This chapter aims to fill in those gaps and uncover if there are any differences in response between plants at different stages of development.

5.2 Materials and methods

120 wheat seeds of the variety Mulika (Senova Seeds) were sown 1 inch deep into 5 inch pots containing John Innes number 3 compost on the 8th of June 2017. A further 120 seeds were sown on the 22nd June and an additional 120 were sown on the 6th of July 2017. The sowing of seeds in these 3 batches, each with a two-week gap, ensured that plants would be two-, four- and six-weeks post emergence at the beginning of treatment.

Plants were grown in a greenhouse with supplementary heating (20°C day/10°C night) and lighting (10hr duration, from 8am to 6pm) and watered daily at the base of the plant so as not to disturb aerial parts. After 2 weeks of growth, each batch of plants was inspected and un-germinated seeds, plants with poor growth and plants with overly excessive growth were discarded, so that all plants within a given batch were of a roughly even size. When plants had 3 emerged tillers, the first tiller that had emerged, the main tiller, was tagged with a small piece of wool so that it could be identified later.

When the plants reached 2, 4 and 6 weeks post emergence (for growth stages of these plants, see Tables 5 and 6), plants from each of the three batches were randomly distributed into six groups of 10 plants for each age. These groups were then assigned as either treated or untreated, so that each age group had 3 groups of treated plants and 3 groups of untreated plants.

Treatment consisted of brushing aerial plant parts with a purpose-built rig (as described in chapter 2) for 20 cycles (one cycle is once forward and once back) once per day for 4 weeks.

Initially the bar was set to 6 cm and raised as the plants grew to ensure the bar brushed plants at half canopy height. Untreated plants received no mechanical treatment but were grown alongside the treated plants. In order to apply treatment, trays containing each group of the plants to be treated were moved from their main location to another bench, where treatment was applied, and then returned to the same place on the main bench. Any damage caused in the process of treatment was noted.

After four weeks of treatment, phenotypic data was collected from each plant including canopy height and tiller count. Plants were then left to continue growing and further measurements were taken when the plants had finished flowering (GS 69), as described in chapter 2. Because of the staggered sowing, these end of flowering measurements for each of the age groups were taken at roughly two-week intervals. The following measurements were taken; main tiller height, number of spikes, main tiller flag leaf length and width, and main tiller internode length and diameter. At this point half of the plants from each group were randomly selected and destructively harvested. Main tiller stems were used for mechanical testing, while the remaining plant material was oven dried at 60°c for 48 hours.

Final measurements were taken when all remaining plants (5 plants per group) had reached maturity and were fully senesced. Each spike was measured for length, main tiller spikes were tagged, and all spikes from each plant were cut 1cm from the base of the spike and removed. All spikes from each plant were then weighed as well as the individual main tillers. From this, average spike weight per plant was calculated. The remaining above ground material was cut at the soil surface, removed and weighed to determine above ground biomass. Main tillers were separated and weighed again separately, internode length and diameter measurements

taken, and kept for mechanical testing. The remaining biomass from each plant was then processed by separating leaves from stem material after which the stem material was milled using a hammer mill.

A selection of plants was destructively harvested at the end of flowering and the main tillers used for mechanical testing as described in chapter 2. The main tillers of senesced plants were also used for mechanical tests, while remaining materiel was used for enzymatic saccharification tests. Spikes from the main tillers of senesced mature plants was imaged using micro CT scanning and data produced on grain size, shape and number.

Mechanical tests, grain CT scanning and image processing, data analysis and statistical processing were conducted as described in the materials and methods of Chapter 2.

5.3 Results

Treatment began at 2, 4, or 6 weeks after seed emergence and will be abbreviated to 2w, 4w, and 6w, respectively.

5.3.1 Age and growth stages of plants

Tables 3 and 4 detail plant age and growth stage at each stage of the experiment.

Table 5 - Age of plants in number of weeks after germination at each timepoint

	T0	**T1**	**T2**	**T3**
Time point	start of treatment	End of treatment	End of flowering	Maturity
Plant age in weeks post germination	2	4	12	24
	4	8	12	24
	6	10	12	24

Table 6 - Growth stages of each age group at each time-point when data was collected

	Age at start of treatment	**T0**	**T1**	**T2**	**T3**
Approximate growth stage	2 weeks (2w)	Tillering	Flag leaf emerged	GS 69 End of flowering	GS93+ Maturity – Plants fully senesced
	4 weeks (4w)	Flag leaf emerging	Ear emerging		
	6 weeks (6w)	Ear emerging	Flowering halfway		

5.3.2 Height

Plant height was measured at the end of treatment from the soil surface to the tip of the highest point of each plant. This measurement included any flowering spikes. At the end of flowering, the height of main tillers only was recorded from the soil surface to the base of the flowering spike. Main tillers consistently consisted of four internodes and the length and diameter of each internode on the main tiller was measured.

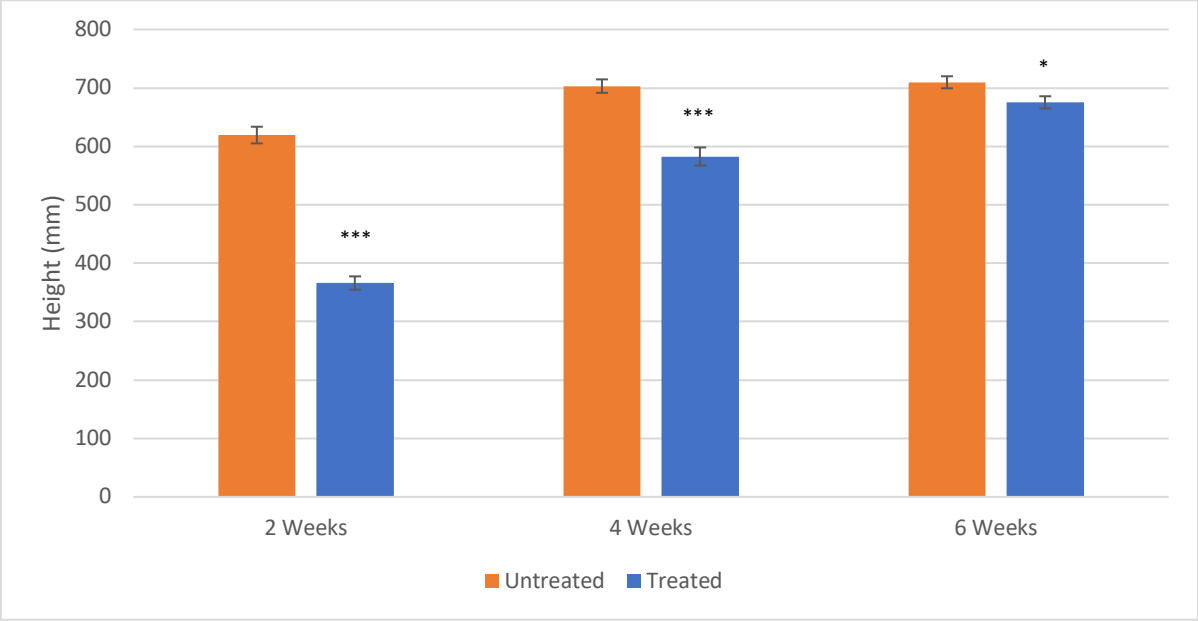


Figure 50 - Plant height at the end of treatment (T1). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=30.

When height was measured at the end of treatment (T1), all treatments resulted in a reduction in plant height (results shown in Figure 50). It can be seen here that older plants were successively taller than younger ones, while there was also a noticeable decrease in the difference between treated and untreated plants. For both 2w and 4w plants the difference in height between treated and untreated plants was highly significant, with a t-test indicating $p < 0.001$, while the difference in height for 6w plants was less significant with $p < 0.05$.

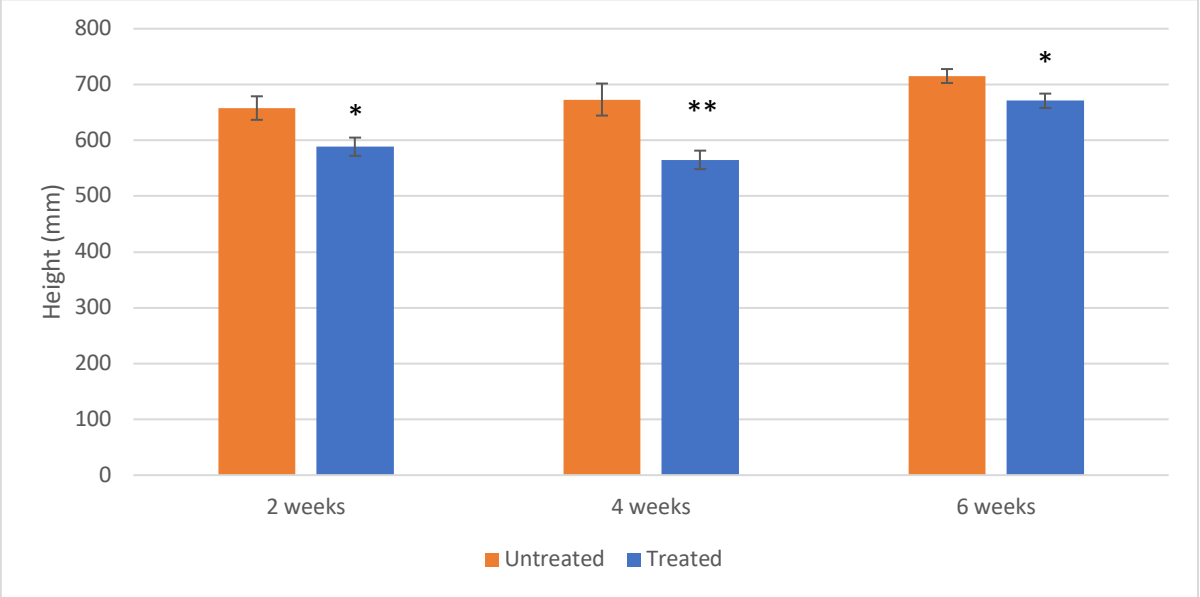


Figure 51 - Main tiller height at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=20

When main tiller height was measured after flowering (Figure 51), the greatest difference in height (108 mm on average) was between treated and untreated plants where treatment started at four weeks post emergence ($p < 0.005$). The difference between treated and untreated main tiller heights for 2w and 6w plants was also significant ($p < 0.05$), but smaller (69 mm and 44 mm respectively).

5.3.3 Internode measurements

At the end of flowering, the length of each internode on the main tiller was measured from bottom-most internode (1) to the top (4).

*Table 7 - Main tiller internode lengths (mm) at end of flowering (T2), from bottom (internode 1) to top (internode 4). ± Standard deviation. Stars indicate level of significance – * = p<0.05, ** = p<0.005, *** p<0.001.*

Internode Length (mm)		Internode 1	Internode 2	Internode 3	Internode 4
2 Weeks	Untreated	61.8 ± 14.52	102.3 ± 10.47	159.1 ± 15.43	227.1 ± 67.18
	Treated	29.2 ± 18.15	68.4 ± 22.59	138.3 ± 25.34	227.7 ± 63.17
		p<0.001	p<0.001	p<0.001	p = 0.390
		***	***	***	
4 Weeks	Untreated	54.8 ± 21.88	104.7 ± 15.10	179.2 ± 18.28	228.7 ± 67.60
	Treated	47.6 ± 15.35	87.1 ± 17.48	126.6 ± 25.81	190.3 ± 59.25
		p = 0.163	p<0.001	p<0.001	p = 0.004
			***	***	**
6 Weeks	Untreated	48.2 ± 23.57	98.8 ± 11.33	158.8 ± 17.10	268.7 ± 74.62
	Treated	55.0 ± 20.99	103.4 ± 13.56	139.1 ± 29.23	247.8 ± 51.67
		p = 0.375	p = 0.159	p = 0.001	p = 0.041
				**	*

Treatment significantly reduced internode length of the first three internodes of 2w plants, the last three internodes of 4w plants and only the last two internodes of 6w plants (see Table 7).

*Table 8 - Main tiller internode diameter (mm) at end of flowering (T2), from bottom (internode 1) to top (internode 4). ± Standard deviation. Stars indicate level of significance – * = p<0.05, ** = p<0.005, *** p<0.001.*

Internode diameter (mm)		Internode 1	Internode 2	Internode 3	Internode 4
2 Weeks	Untreated	2.99 ± 0.171	3.84 ± 0.106	4.23 ± 0.165	2.75 ± 0.393
	Treated	2.58 ± 0.347	2.88 ± 0.209	3.25 ± 0.195	2.56 + 0.367
		p = 0.003	p < 0.001	p < 0.001	p = 0.262
		**	***	***	
4 Weeks	Untreated	3.00 ± 0.291	3.56 ± 0.296	3.99 ± 0.198	2.83 ± 0.274
	Treated	2.90 ± 0.253	3.41 ± 0.202	3.66 ± 0.387	2.47 ± 0.350
		p = 0.169	p = 0.024	p = 0.001	p < 0.001
			*	**	***
6 Weeks	Untreated	3.10 ± 0.319	3.52 ± 0.203	3.77 ± 0.234	2.77 ± 0.225
	Treated	3.14 ± 0.270	3.60 ± 0.272	3.80 ± 0.198	2.69 ± 0.192
		p = 0.626	p = 0.191	p = 0.632	p = 0.323

Mechanical treatment generally reduced the diameter of wheat stems across all internodes (Table 8), apart from 6w plants where there was no significant difference in the diameters of internodes between treated and untreated plants. Internode diameter in 2- and 4w treated plants followed a similar pattern as measurements for internode length; internodes 1, 2, and 3 are significantly affected by treatment of 2w plants, while internodes 2, 3, and 4 of 4w plants were significantly affected. In particular, the effect on internodes 2 and 3 for 2w plants is notable, with a decrease in diameter of 25% and 23%, respectively, compared with untreated controls.

5.3.4 Tillers and flowers

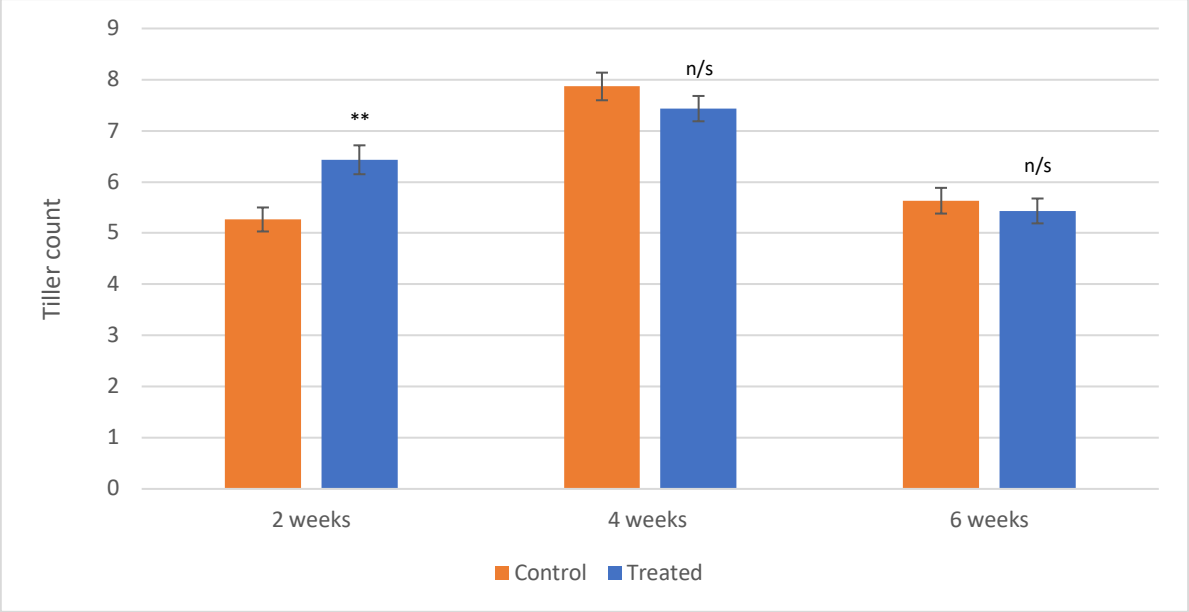


Figure 52 - Tiller count at the end of treatment (T1). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=30.

The number of tillers produced by each plant was counted after treatment had ceased (results shown in Figure 52). As seen in the previous chapter, brushed plants have an increased number of tillers, when treatment starts at 2 weeks post emergence. However, this trend does not continue for plants where treatment started later. Plants treated after 4 and 6 weeks

both have fewer tillers than their related untreated groups. While the difference between treated and untreated 2 weeks post emergence is significant (ANOVA $p < 0.01$), there is no significant difference in tiller count for either 4w or 6w groups. By the end of treatment, 4w plants (both treated and untreated) had more tillers than 2w and 6w plants.

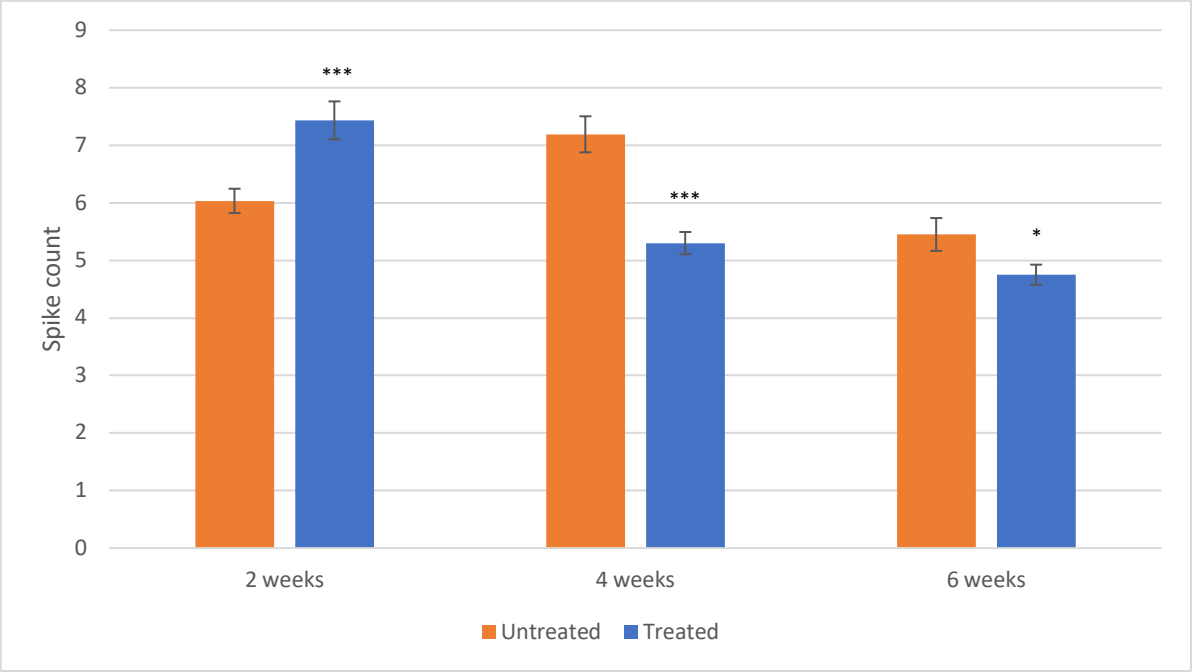


Figure 53 - Average number of flowering spikes per plant at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=20.

At the end of flowering, the number of flower-bearing tillers (flowering spikes) was counted (Figure 53). It is most noticeable that when treatment began at 2 weeks post emergence, significantly more flowers were produced per plant ($p < 0.01$). However, when treatment began at both four- and six-weeks post emergence, fewer flowering tillers were counted at the same time-point, with the difference between treated and untreated plants being greatest for 4 weeks ($p < 0.001$). There was no significant difference in the number of flowering spikes for 6w plants.

5.3.5 Mechanical measurements

5.3.5.1 Cross-section area at the end of flowering (T2)

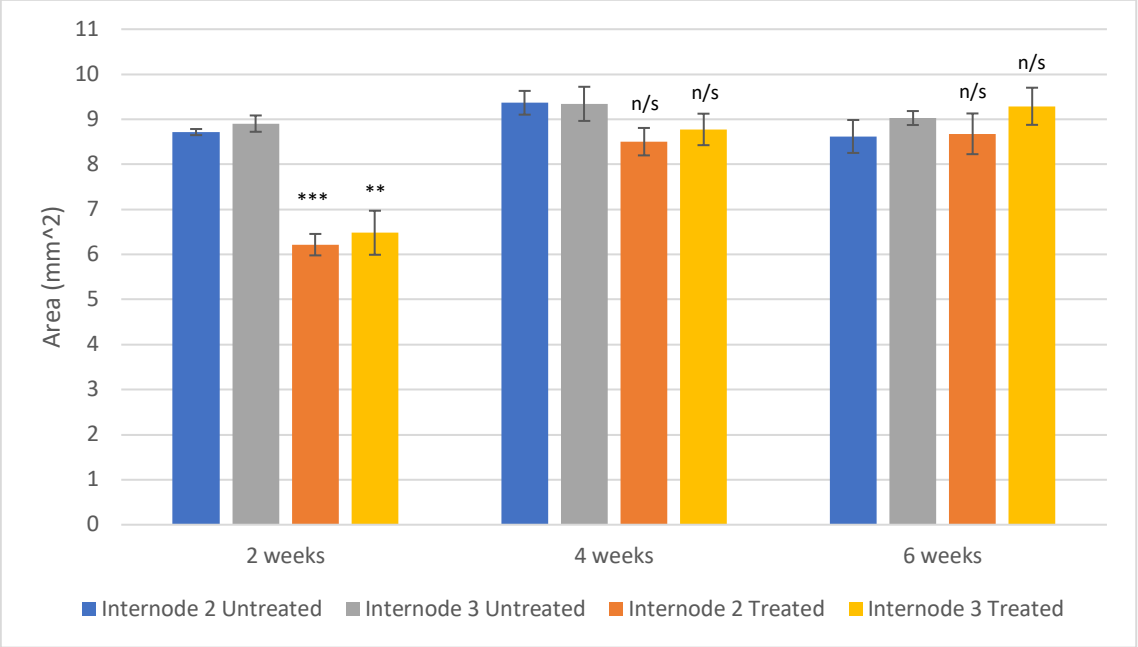


Figure 54 - Cross-section area of internodes 2 and 3 at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=8.

Internodes used in the 3-point bending tests were stored in 70% ethanol prior to sectioning. A free-hand cross section was cut using a razor blade near to the site of impact from the 3-point bending hammer. Sections were photographed under a microscope and images were analysed using ImageJ to determine inner and outer stem diameter and cross-sectional area. Internodes 2 and 3 of plants treated both 2W and 4W after emergence had a lower cross-sectional area compared with untreated plants, whereas treatment at 6 weeks resulted in a slight increase in area. The difference in cross sectional area between treated and untreated 2W plants was highly significant for both internodes ($p<0.001$). On average, the second internode of treated 2w plants was 2.5 mm^2 less than untreated, while the difference for the third internode was 2.4 mm^2 (Figure 54). Treated 4w plants had a 0.9 mm^2 reduction in the area of internode 2, and a reduction of 0.6 mm^2 for internode 3, though these differences were not significant.

5.3.5.2 Bending rigidity at T2

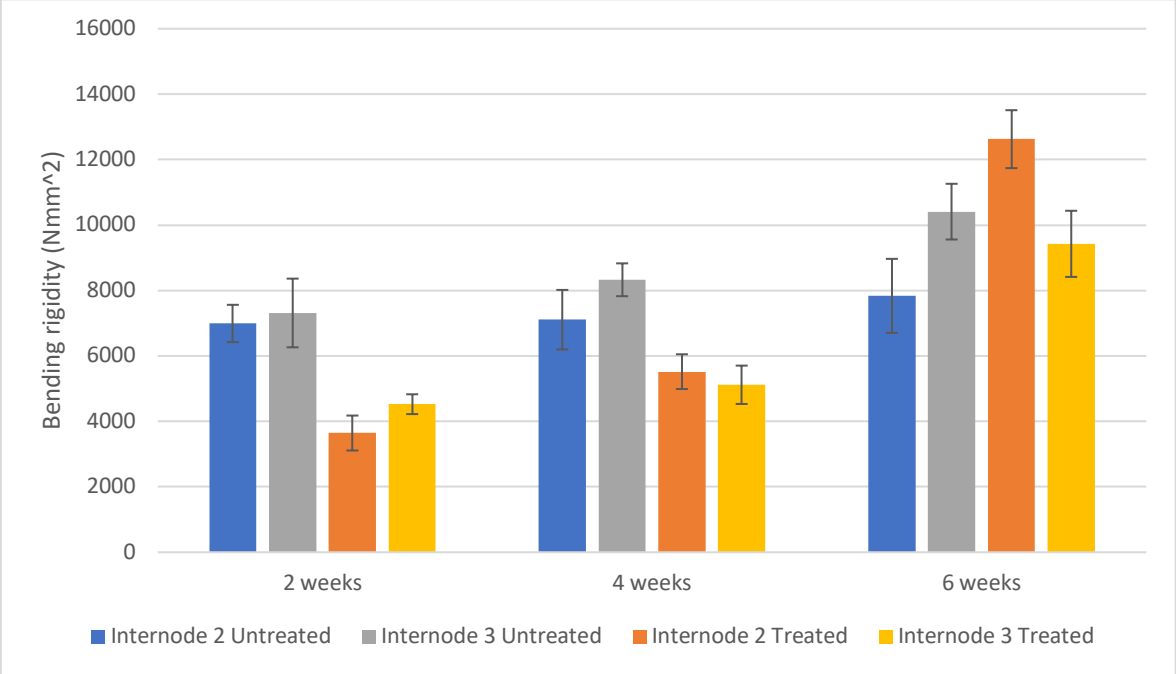


Figure 55 - Bending rigidity of internodes 2 and 3 at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=8.

Treatment resulted in a reduction in the bending rigidity of the second internode of both 2W and 4W plants, while the second internode of 6W treated plants saw an increase (Figure 55). The second internode of 2W treated plants had a 48% reduction in bending rigidity compared with untreated plants, while 6W plants had a 38% increase. The lower internodes of both 2W and 6W plants were significantly affected by treatment, $p<0.01$. Following treatment, there was a decrease in bending rigidity of the third internode across all three age groups, however the effect was only significant for 4W plants, $p<0.001$.

5.3.5.3 Bending strength at T2

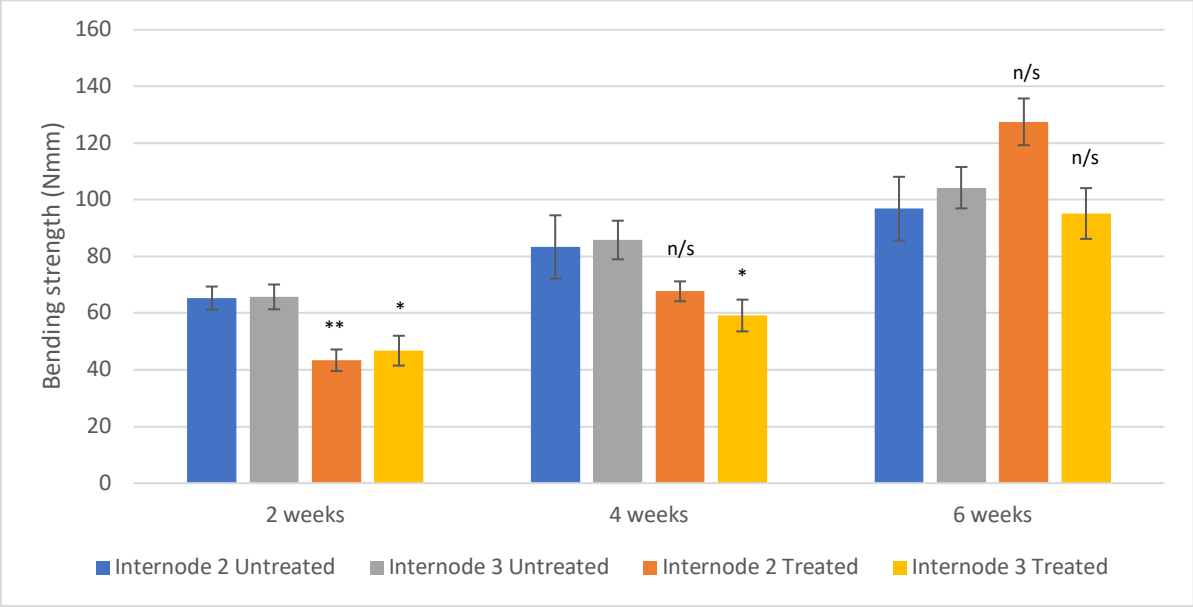


Figure 56 - Bending strength of internodes 2 and 3 at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to untreated plants according to a t test. n=8.

Internode bending strength followed a similar pattern to the bending rigidity (see Figure 56); the second internode of 2W and 4W treated plants had a reduced bending strength, while there was an increase for 6W treated plants. For internode 3, all age groups had a reduced bending strength compared with untreated plants. Treatment had a significant effect on both internodes of 2W plants ($p<0.01$ for internode 2 and $p<0.05$ for internode 3), and also on the third internode of 4W plants ($p<0.01$). The increase in the bending strength of the second internode of 6W plants was not significant.

5.3.5.4 Young's modulus at T2

The second internode of treated plants had an increased Young's modulus across all age groups. However, only 2W treated plants had an increase in the Young's modulus of the third internode, while there was a decrease for both 4W and 6W treated plants (Figure 57). The greatest difference in young's modulus of the second internode was for 6W plants, with 0.415

GPa between treated and untreated plants, while the difference for 4W was 0.133 GPa and only 0.036 GPa for 2W plants. The increase in Young's modulus seen in 2W treated plants (0.221 GPa) was less than the decrease for 4W treated plants (0.251 GPa). An analysis of variance test indicated there was no significant difference in Young's modulus between treated and untreated internodes 1 and 2 for all age groups.

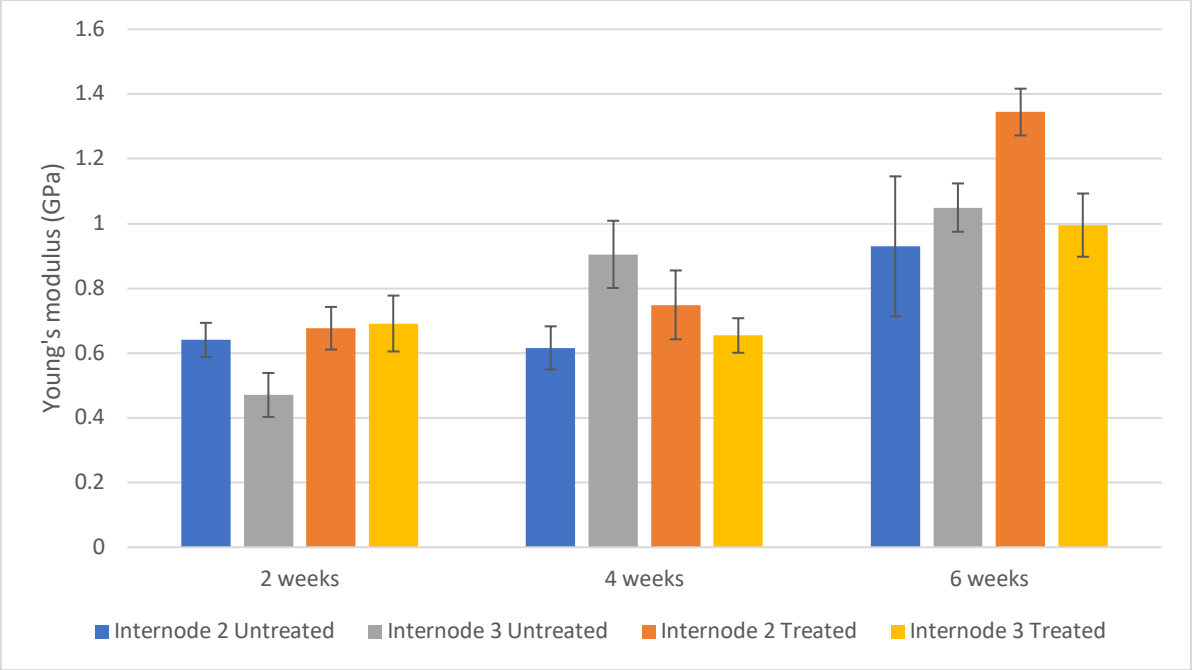


Figure 57 - Young's modulus of internodes 2 and 3 at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. n=8.

5.3.5.5 Outer diameter of senesced mature plants (T3)

At T3 the mature plants had become completely senesced and only the third internode was measured. The outer diameter of internode 3 of senesced stems was measured using ImageJ and digital images of cross-sections (Figure 58). Treatment significantly reduced the outer diameter of 2W plants ($p<0.001$) with no significant differences in diameter between the treatments of 4W and 6W plants.

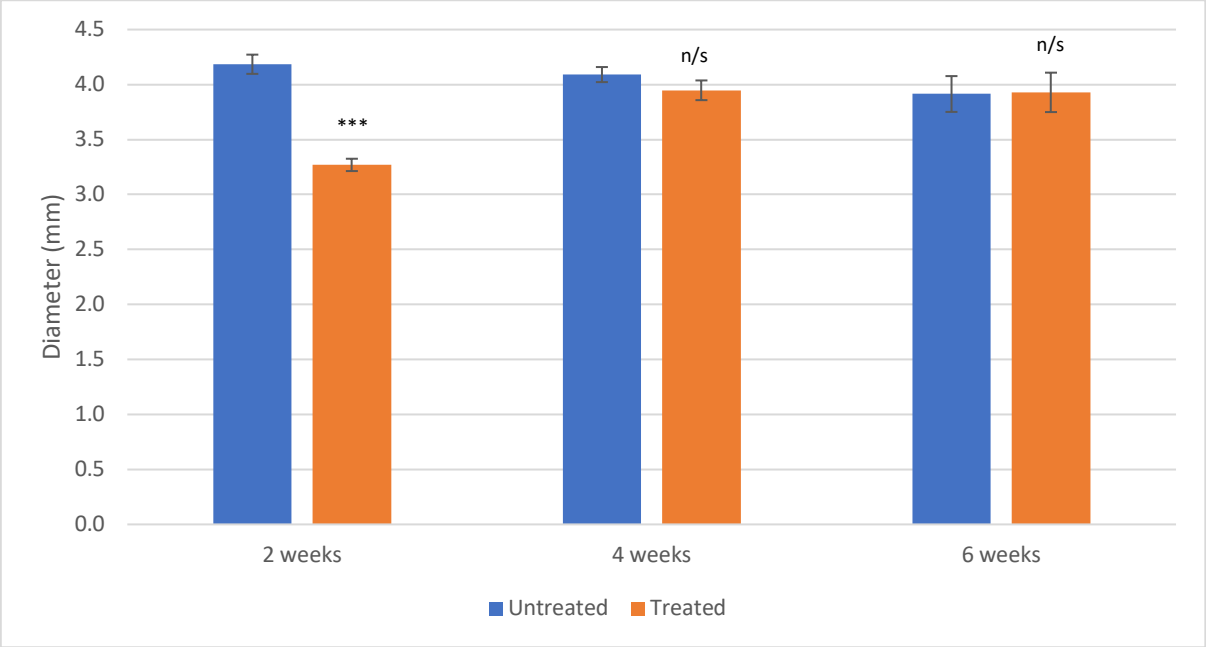


Figure 58 - Outer stem diameter of the third internode senesced mature plants harvested at T3. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=7.

5.3.5.6 Cross-section area at T3

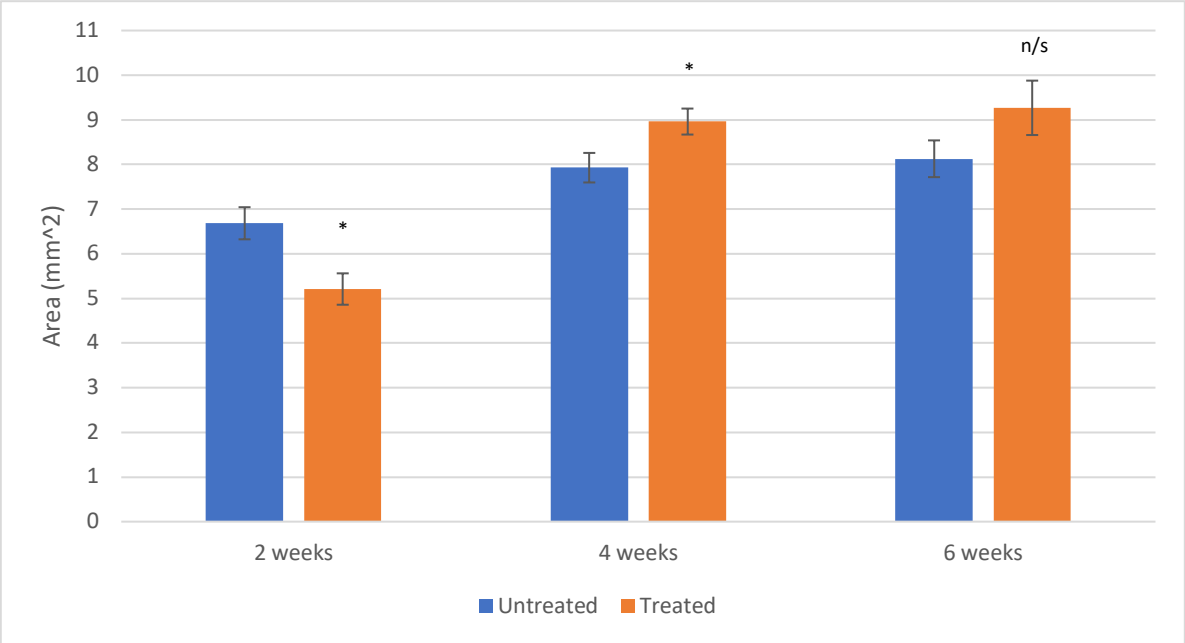


Figure 59 - Cross-section area of the third internode of senesced mature plants harvested at T3. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=7.

Cross-section area was calculated using multiple measurements of the stems outer and inner radius, where stems were hollow, and just on the outer radius for filled sections.

For 2W plants, treatment reduced cross-section area by 1.47 mm^2, while treated 4W and 6W plants had greater cross-sectional areas (an increase of 1.03 mm^2 and 1.14 mm^2, respectively), shown in Figure 59. An analysis of variance indicated significant differences between treated and untreated 2W and 4W plants, $p < 0.05$.

5.3.5.7 Bending rigidity at T3

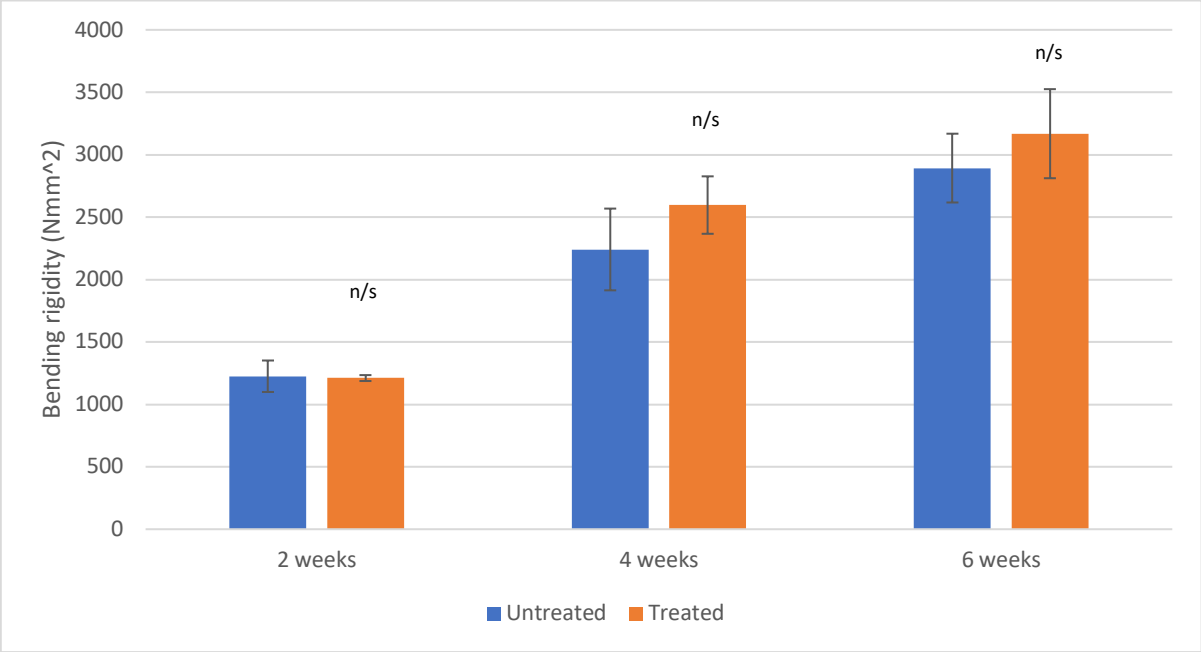


Figure 60 - Bending rigidity of the third internode senesced mature plants harvested at T3. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=7.

2W plants were unaffected by treatment while treated 4W and 6W had a greater bending rigidity than untreated plants, though none were found to be significantly different (Figure 60).

5.3.5.8 Bending strength at T3

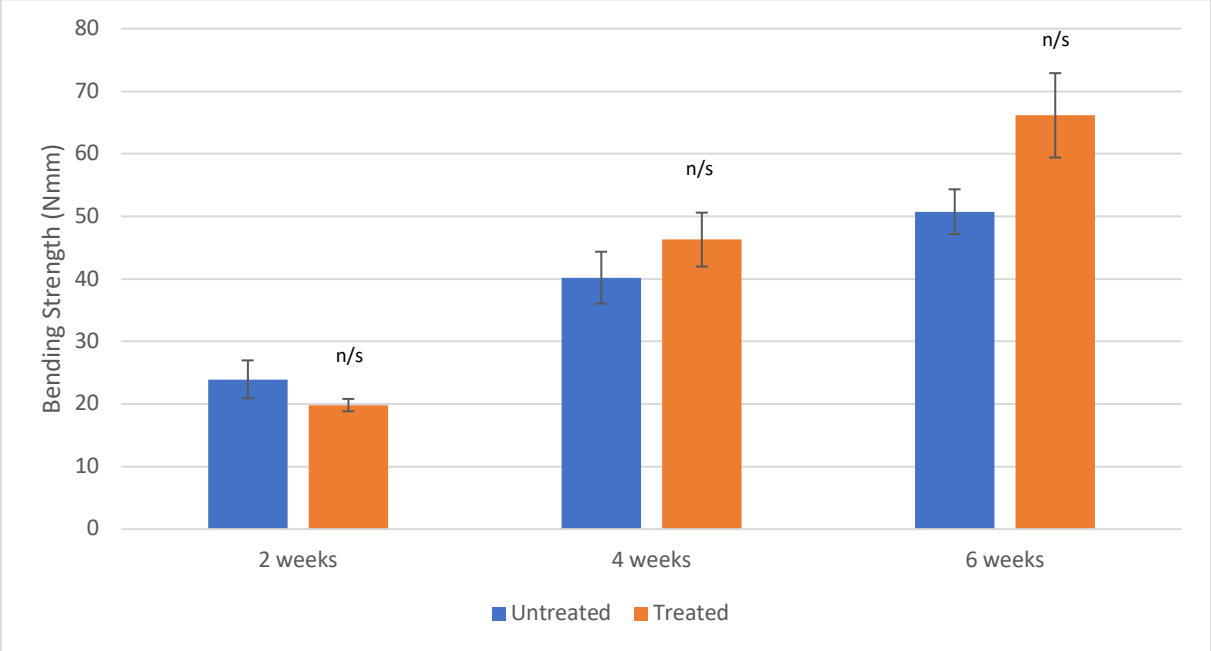


Figure 61 - Bending strength of the third internode senesced mature plants harvested at T3. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=7.

Treatment reduced the bending strength of the third internode of 2W plants, while there was an increase in the bending strength of treated 4W and 6W plants (Figure 61). 6W plants had the greatest difference in bending strength between plants that had received treatment and plants that had not, 15.40 Nmm, whereas the difference for 4W plants was 6.09 Nmm and 2W plants saw a 4.14 Nmm increase. An analysis of variance indicated no significant differences between treated and untreated plants across all age groups.

5.3.5.9 Young's modulus at T3

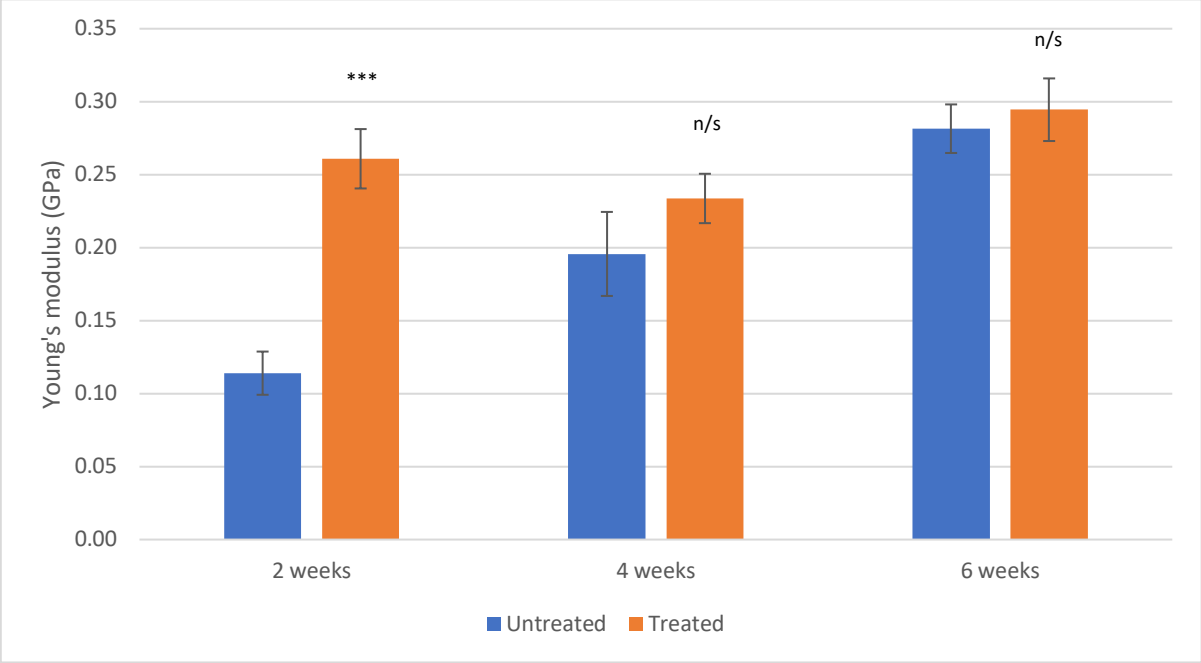


Figure 62 - Young's modulus of the third internode senesced mature plants harvested at T3. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=7.

Plants that received treatment had a higher Young's modulus than plants that were untreated, across all age groups, though treatment of 2W plants had the greatest effect, increasing Young's modulus by 56%. 6W plants were the least affected by treatment (Figure 62). Only 2W plants were significantly affected by the treatment, $p < 0.001$.

5.3.6 Spike measurements and grain yield

5.3.6.1 Main tiller spike weight

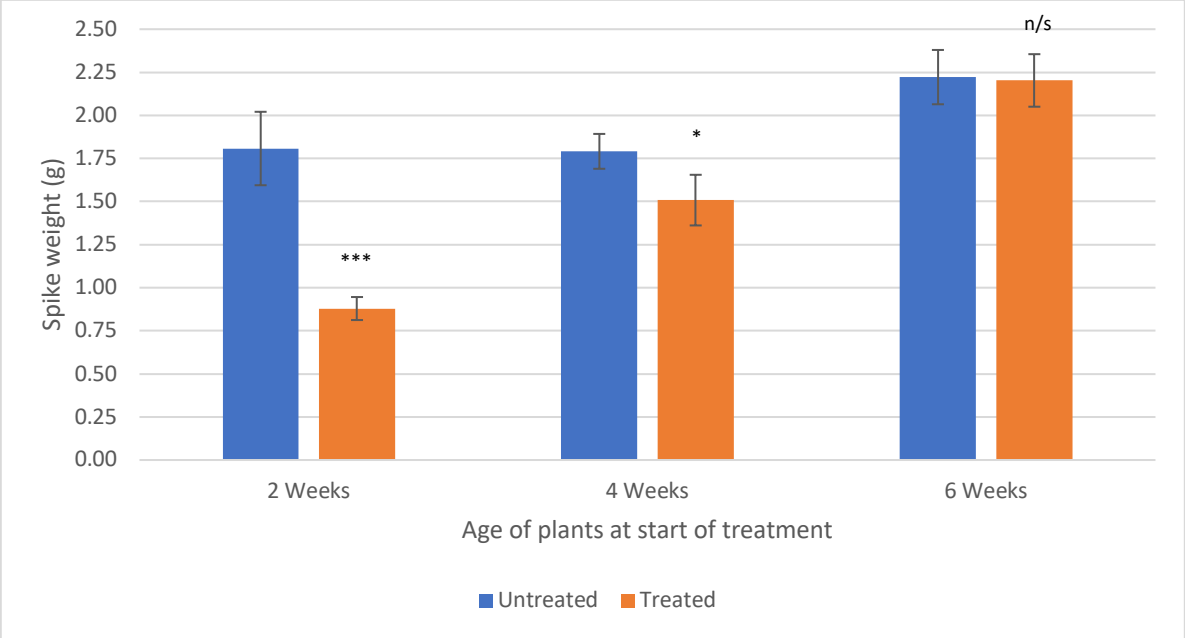


Figure 63 - Weight of mature main tiller spikes (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=10.

Spikes were removed from the main tiller and weighed to the nearest 0.001g. Treatment significantly reduced main tiller spike weight for 2W plants ($p < 0.001$), and 4w plants ($p = 0.045$), while there was no difference in spike weight between treated and untreated 6w plants (Figure 63). On average, spikes from treated 2W plants weighed 0.929g less than those from untreated plants, while the average weight of spikes from treated 4w plants was 0.283g less.

5.3.6.2 Main tiller spike length

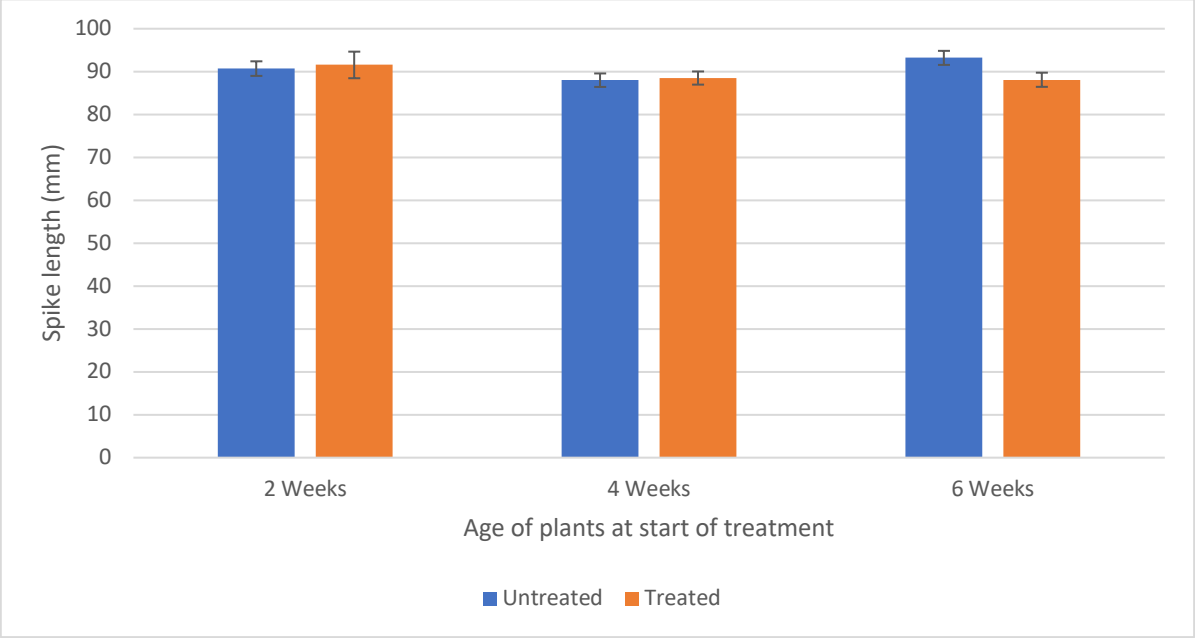


Figure 64 - Length of mature main tiller spikes (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=10.

Each main tiller spike was measured from its base to the top-most point, excluding any awns. There was no difference in spike length between treated and untreated plants of both 2W and 4W plants, however treated spikes from 6w plants were on average 5.1 mm shorter than those from corresponding untreated plants ($p = 0.042$) (Figure 64).

5.3.6.3 *Total spike weight per plant*

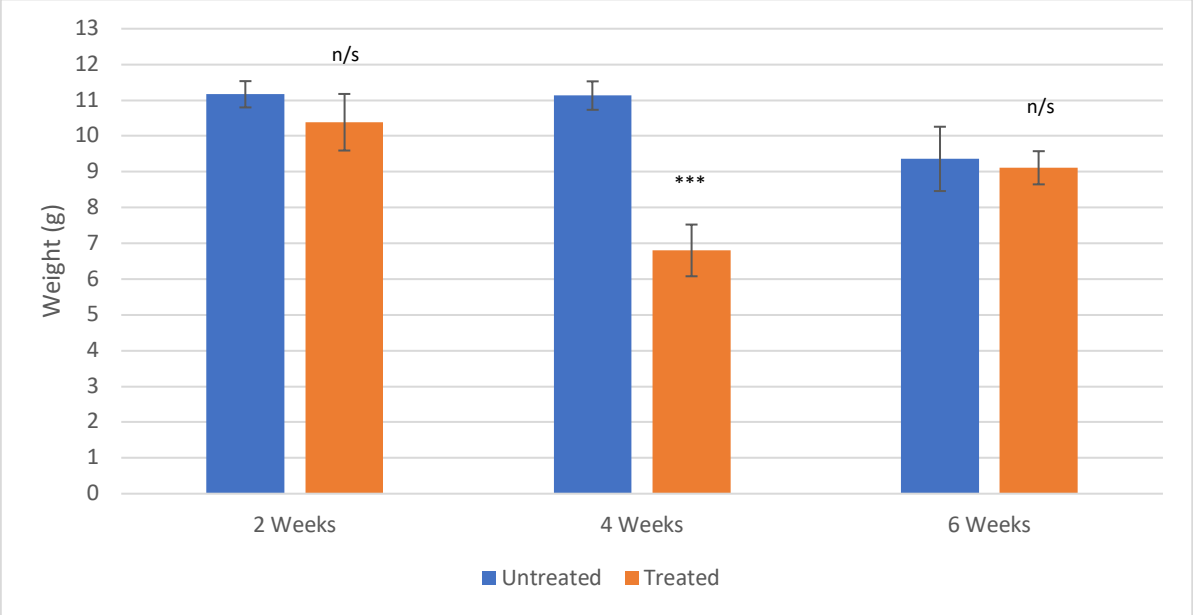


Figure 65 - Mean total spike weight (weight of all spikes from each plant) at maturity when plans had become senesced (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=10.

All spikes from each plant were separated from the rest of their stems and weighed together to determine total spike weight for each plant. Treatment resulted in a reduction in total spike weight for all age groups (See Figure 65). There was a significant difference ($p < 0.001$) between treated and untreated 4W plants (4.329g reduction). The difference between treated and untreated plants was too small to be significant for 2W and 6W plants, where the difference between untreated and treated total spike weight was 0.782g and 0.249g, respectively.

5.3.6.4 *Average spike weight per plant*

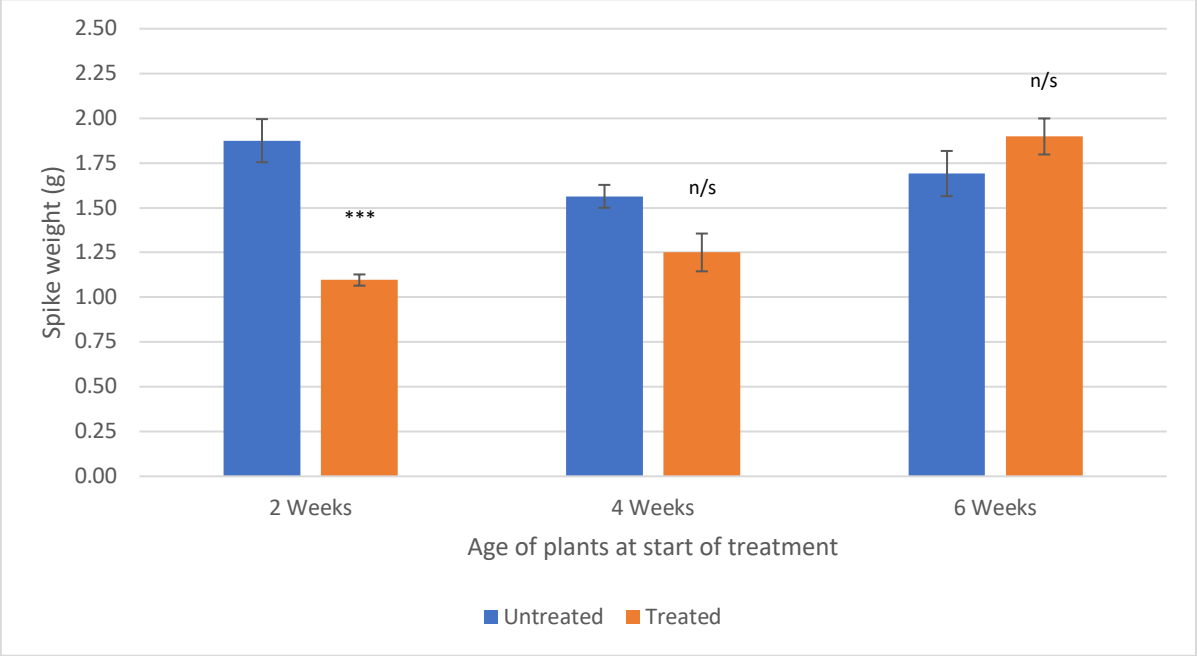


Figure 66 - Mean individual spike weight at maturity when plans had become senesced (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=10.

The average spike weight per plant was determined by dividing the total spike weight with the number of heads on the plant. While treatment reduced the average spike weight for both 2W and 4W plants (0.779g and 0.313g, respectively), treatment appeared to result in an increased spike weight (0.207g) for 6W plants (Figure 66). However, only 2W plants had a significant difference in individual spike weight ($p < 0.001$) between treated and untreated plants.

5.3.6.5 *Individual grain volume*

Treatment significantly reduced individual grain volume of 2W treated plants, $p < 0.001$ and 4W plants $p < 0.01$ (see Figure 67). When treatment started at 6 weeks post emergence, an 11% increase in individual grain volume was seen, significantly larger than untreated plants, $p < 0.01$.

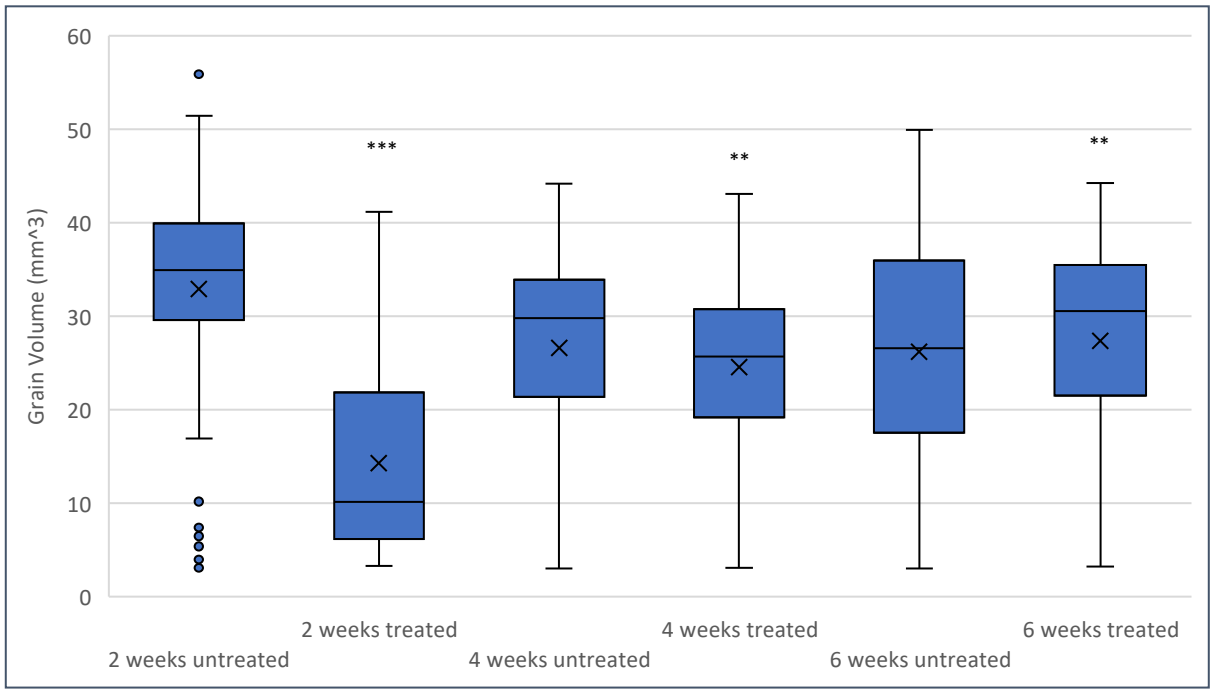


Figure 67 - Individual grain volume in main tiller spikes (T3). In this chart, the upper whisker indicates the top 25% of data, excluding outliers, and the lower whisker indicates the lowest 25% of data, excluding outliers which are marked as dots beyond the whiskers. The X indicates the sample mean and the median value is indicated by the line inside the box. The box itself indicates the interquartile range, where 75% of measurements fall.

5.3.6.6 Grain count

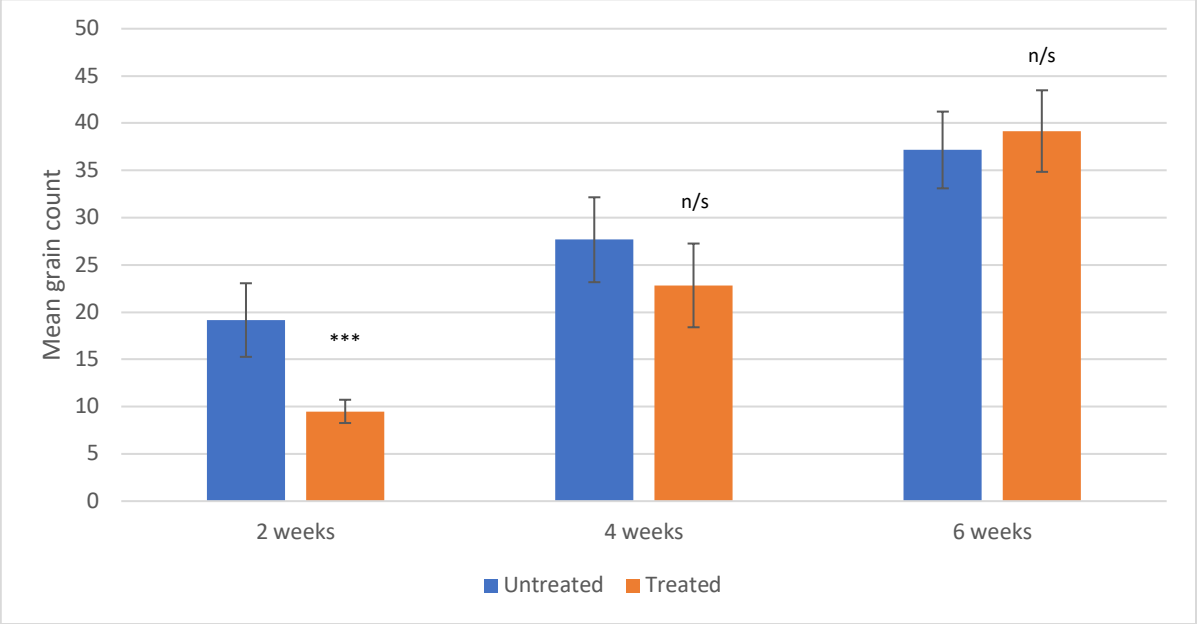


Figure 68 - Mean number of grains in main tiller spikes (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=6.

Treatment of 2W plants resulted in a significant reduction in the number of grains per main tiller spike ($p < 0.05$), but treatment did not significantly affect 4W plants (Figure 68). There was an increase in individual grain volume of 2.7 mm^3 for treated 6W plants, though not a significant increase.

5.3.6.7 Total grain volume

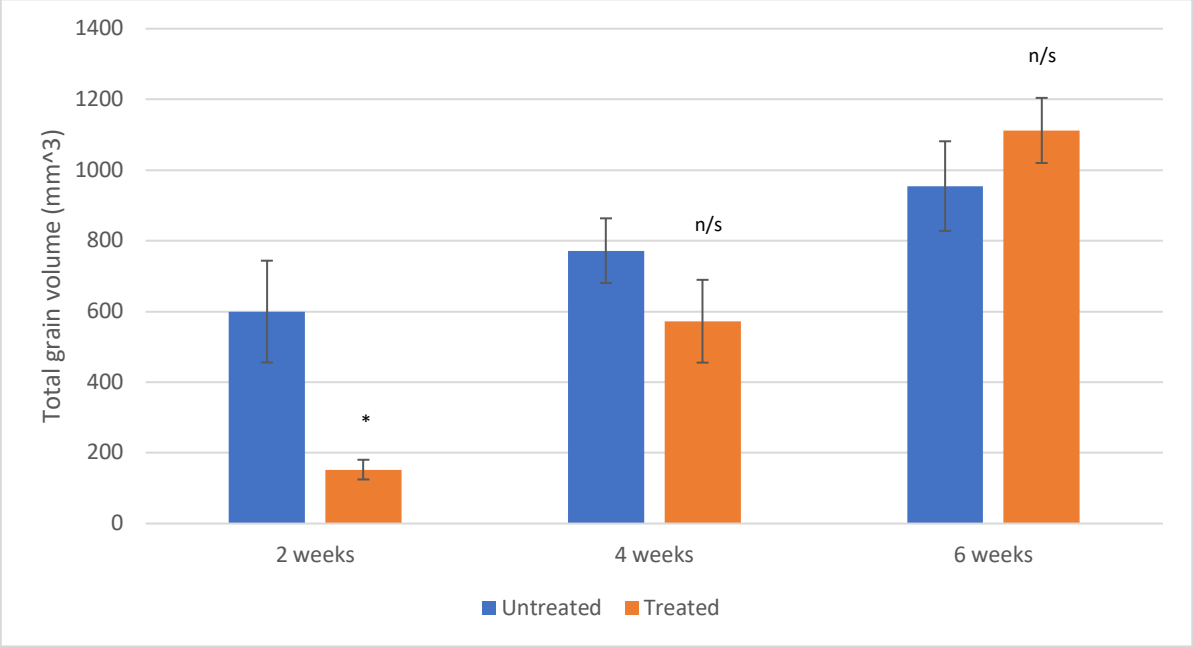


Figure 69 - Total volume of grains in each main tiller spike (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=6.

Individual grain volume of all the grains in each head was totalled to give a total grain volume for each main tiller spike. 2W plants had an average total grain volume of 152.6 mm^3, while the total grain volume of untreated spikes was 599.7 mm^3 (Figure 69). Treatment of 2W plants resulted in a significant reduction in total grain volume $p < 0.05$, though there was no significant difference for either 4W or 6W treated plants.

5.3.7 Above ground biomass

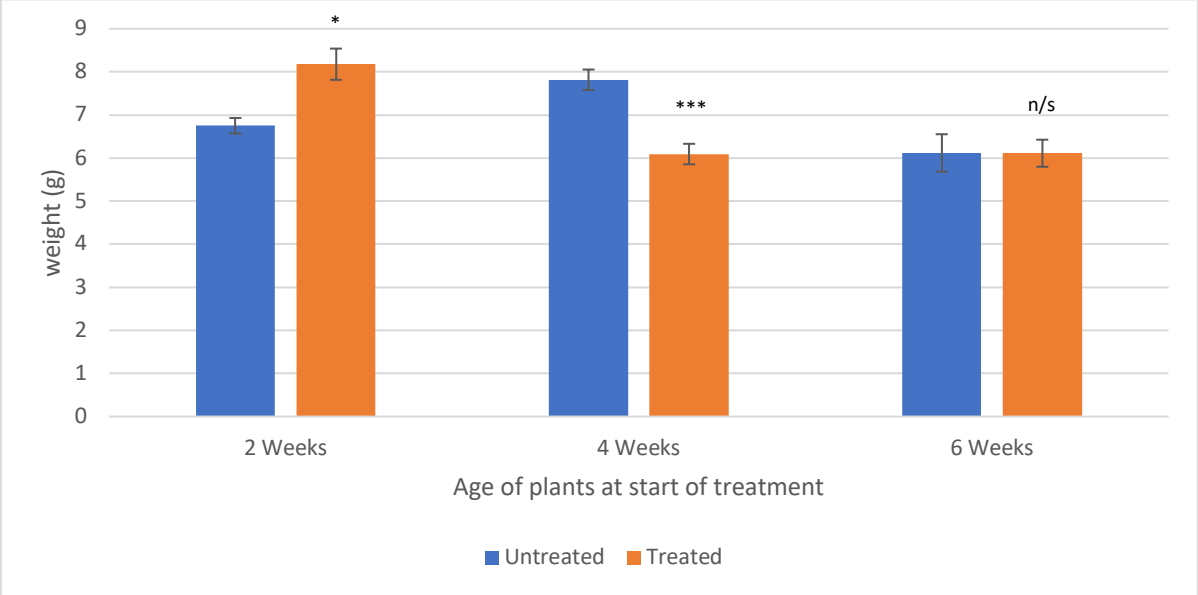


Figure 70 - Above-ground biomass of senesced mature plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. n=10.

After all spikes had been removed, the remaining above ground material was separated from the root mass at soil level and weighed to determine aboveground biomass. Treatment increased the aboveground biomass for 2W plants (1.132g increase) but resulted in a decrease for 4W plants (1.859g decrease), while there was no difference for 6W plants (see Figure 70). Differences between treated and untreated groups were significant for both 2W and 4W ($p = 0.015$ and $p < 0.001$, respectively).

5.4 Discussion

5.4.1 Tillers and flowering

At both the beginning and end of treatment, the three age groups were at different growth stages as detailed in Table 5 and 6. By the end of treatment, 2W plants had an emerged flag leaf on the main tiller, while the ears of 4W plants were starting to emerge and 6W plants were halfway through flowering. At this point, 2W plants were potentially still producing tillers, while 4W and 6W plants had already reached their peak for tiller numbers. Between tillering and flowering, some tillers die off (this process is detailed in chapter 1) though plants with more tillers generally produce more flowers. Stresses and the growth environment may affect tiller numbers and therefore have an effect on final flower number.

Only tiller numbers for 2W plants were significantly affected by treatment, which indicates that plants are most sensitive during early stages of tillering. There was a slight reduction in tiller numbers between treated and untreated 4W plants, which indicates plants may also be sensitive to treatment in later tillering stages and during stem-extension. Treatment at this time may be influencing tiller death or flowering spike formation, though it is hard to know without more specific study.

Zhao et al, (2018) noticed that rubbing rice plant stems led to a 21% and 23% increase in tiller numbers, and external stimuli such as mechanical stimulation can influence tiller numbers. There is a lack of further information as to how and why mechanical treatment may increase tiller numbers. However, the effect of other stresses, such as drought has been studied in wheat and an increase in tillering in drought experiments has previously been observed. Blum

et al. (1990) noticed a very high rate of tiller appearance upon recovery of wheat plants from drought stress. As tillers were recorded upon appearance (rather than initiation), it was suggested that early stress did not stop tiller initiation, while it did halt tiller development. Therefore, when stress was removed, treated plants were observed to produce more tillers. Shah and Paulsen (2003) observed that when wheat plants were treated with a similar drought stress during anthesis, tiller numbers remained constant between treatment and control plants. No difference was seen as the final tiller number was likely to have already been established when treatment began. Though care should be taken to draw direct parallels, there may be similar underlying mechanisms in the response of wheat plants to mechanical stress.

Flowering spike numbers reflected tiller numbers across all age groups and treatments. 2W treated plants had more flowering spikes than untreated, while both 4W and 6W treated plants both had fewer spikes than their associated controls.

Between T1 (end of treatment) and T2 (end of flowering) there was an overall increase in spike numbers compared with tillers for 2W treated plants, while both 4W and 6W plants had a reduction in spike numbers compared with tillers. 6W plants, both treated and untreated, lost very few tillers between T1 and T2, while 4W plants lost significantly more. This suggests that while treating plants during early tillering stages increases tillering, which leads to an increased number of flowers, treating plants during later stem extension stages results in increased tiller loss and an overall reduction in the number of flowering spikes per plant. However, when treatment is applied to plants during flowering, there is little impact on the number of flowering spikes.

5.4.2 Height

After four weeks of treatment, plant height was measured from the soil surface to the top of the highest part of each plant. For 6W plants, the top of the flowering spike was the highest point, while for 4W and 2W plants it was the top of the flag leaf. At this point, plants were 6, 8, and 10 weeks post emergence and therefore at different stages of development, ad described in tables 1 and 2.

2W treated plants were significantly affected by treatment and a noticeable reduction in height could be seen in treated plants compared with untreated. 4W plants were less severely affected by the treatment, but there was still a significant difference between treated and untreated plants. The height difference for 6W plants was less, but still noticeable. These results indicate that plants are most sensitive to mechanical treatment during early stages of development, but also in later stages of stem extension.

Results on height reduction here are consistent with those reported in chapter 2, but also with other studies on the effect of mechanical treatment on wheat plants (such as Crook and Ennos, (1996)). However, there are no other studies which have looked at the effect of applying mechanical treatment to plants at different stages of development specifically.

Once treatment had finished and T1 measurements taken, plants were left to grow in situ until plants reached GS69 (end of flowering), when plant height was measured again. For each plant, main tiller height was measured from the surface of the soil to the base of the floret. As the tree age groups flowered at different points, data was collected on three different

days, approximately 12 weeks post seedling emergence. Plant height at this point reflects the effect of treatment and recovery.

Plant height was reduced across all age groups, with 4W plants having the greatest difference between treated and untreated plants, and 6W plants the smallest. Differences in plant height at T2 are related to how treatment affected stem extension and subsequent effects on internode length.

5.4.3 Internode lengths T2

There was a notable progression in internode response of each age group to mechanical treatment. For the youngest plants, the first three internodes were reduced in height, but internode four was longer for treated plants. There was a small reduction in internode one in treated 4W plants, though the greatest and most significant differences was seen in internodes two, three and four. For 6W plants, the lower two internodes of treated plants were longer, while internodes three and four were significantly shorter than controls.

These patterns are linked to the progression of internode development of plants during treatment. At the beginning of treatment, 2W plants were only beginning stem elongation, while 4W plants were in the midst of stem elongation stage. 6W plants were beginning to flower, indicating that these plants were likely to be nearing their final height.

It's interesting to note that treatment of the 6W plants appear to increase the length of lower internodes even though it would be expected that these internodes had likely achieved full extension by the time treatment was applied. However, the increase in lower internode

growth was offset by the reduction in the length of upper internodes of treated plants, resulting in an overall reduction in main tiller length.

At the point treatment ceased, 2W treated plants were at flag leaf emergence growth stage and therefore internodes had yet to achieve full extension. The small difference in internode four length indicates that extension was unaffected during treatment, but there may have been a slight bounce-back in growth once treatment had been removed.

5.4.4 Internode diameter

Treatment reduced internode diameter of both 2W and 4W plants but had no significant effect on 6W plants. The pattern of internode response reflects the pattern of internode extension during the period of treatment. The first three internodes of 2W plants were significantly affected by treatment, while there was little difference in the diameter of the fourth internode. For 4W treated plants, the lowest internode was unaffected by treatment, while the effect on the second, third and fourth was significant.

As discussed in chapter three, most studies on the responses of plants to mechanical treatment demonstrated and increase in stem and internode diameter. Whereas, Crook and Ennos (1996) noted a significant reduction in the diameter of free-standing wheat plants compared with supported plants at 30 cm along the stem.

5.4.5 Stem mechanical properties

Treatment reduced both stem diameter and cross-section area of plants when treatment started at 2 weeks after emergence. A reduction in the dimeter of the third internode at the

end of flowering was also seen in chapter 4. This effect remained clear in senesced mature stems. Treatment did not affect the diameter of internode sections from either 4W or 6W plants, though there was a significant increase in cross-section area of 4W plants, which is due to a decrease in the internal diameter of the stem.

Treatment of both 2W and 4W plants appears to reduce stem bending strength when measured at the end of flowering. The pattern of internode response reflects the internode development stages during the period of brushing. Treatment of 2W plants significantly affecting internode two, and 4W plants seeing a greater effect in the third internode. However, there was a surprising increase in the bending rigidity of the second internode of 6W treated plants.

Effects on the bending strength and bending rigidity of 2W plants reflect the results seen in chapter three, though in this case no significant effect on Young's modulus was seen at the end of flowering. A review by Coutand (2010) stated that many studies reported an increase in stem stiffness, but also a decrease in the Young's modulus.

Mechanical properties including bending strength are closely related to the outer stem diameter and stem wall thickness (Wang et al. 2006). A decrease in stem diameter results in a reduction in the second moment of area, and thus a reduction in stem stiffness (Coutand et al. 2000, Kokubo, Kuraishi, and Sakurai 1989). Though it is common for stem strength to decrease with an increase in stem diameter. This indicates that stem thickness is not necessarily the main factor determining mechanical properties (Wang et al. 2006).

These results suggest that young plants are more sensitive to mechanical treatment, and treatment affects the stems mechanical properties. changes in mechanical properties can be seen in the mature plants. Increases in the Young's modulus means that more force is required to bend the stems. This could have implications for stem lodging and requires further study.

5.4.6 Grain yield

As seen in Chapter 4, starting treatment at 2 weeks post emergence reduced individual grain volume and grain count, which resulted in an overall reduction in total grain volume per main tiller. When the start of treatment was delayed to 4 weeks post emergence, there was still a significant reduction in individual grain volume, but no effect on grain count or total grain volume. Starting treatment at 6 weeks resulted in an increase in individual grain volume but had no effect on either grain count or total grain volume.

Reductions in grain volume could be due to re-mobilisation or re-allocation of assimilates away from grain formation or filling, as discussed in Chapter 4. Treatment of both 4W and 6W plants overlapped with grain formation and filling, whereas treatment of 2W plants overlapped with flowering only. This could mean that treatment may be affecting flower fertility or development of the flower. Since wheat is self-pollinating, it could be that the bar of the brushing rig is disrupting or affecting the flower ad it passes over.

Drought stress applied during tillering stages resulted in a reduction in the number of grains per spike, though there was a small effect on grain weight (Blum et al. 1990). When drought stress was applied during grain filling, there was some reduction in grain weight, but effect

on the number of grains per spike (Shah and Paulsen 2003). Foulkes et al. (2010) suggested that stressing plants during grain filling can present a risk to the grain filling process, and this leads to a reduction in grain yield. Shah and Paulsen (2003) suggest that it is unclear whether the decrease in grain weight under stress is due to a reduction in photosynthetic capacity or enzyme processes within the grain. Effects on enzymatic processes may be a problem in heat or drought stressed plants, but it is less likely to be a problem in well-watered but mechanically stressed plants grown at a moderate temperature. Conversely, Zhao et al. (2018) found an increase in photosynthesis and transpiration rates after rubbing the stems of rice plants. Acevedo et al. (2002) suggests that grain yield is more limited by sink size (number of grains) than post-anthesis assimilate supply. Environmental stressed, such as drought and nutritional stress are known to affect floret fertility, therefore reducing grain numbers. There is a significant lack of data on the effect of mechanical stress on grain yield, and further research is needed to understand the reasons for reductions in yield following mechanical stress.

5.4.7 Conclusion and next steps

It is important to consider that the conditions of these experiments do not reflect those of the natural environment. During the whole experiment, including before treatment began, plants were grown in a greenhouse with near to static air flow. This made it possible to study the effects of applying treatment on plants at specific growth stages only, whereas in the natural environment plants are exposed to mechanical treatment from the moment of seedling emergence. Therefore, the next step is to look at the growth and development of plants grown outside, exposed to natural wind conditions, with and without additional brushing treatment.

Chapter 6

6 The effect of wind exposure and brushing on wheat plants grown under natural conditions

6.1 Introduction

The previous three experiments were conducted in a greenhouse under controlled conditions and with almost static airflow. So how would these plants respond to treatment if grown outside under uncontrolled growth conditions, where plants are exposed to ambient wind? Goodman and Ennos (1997) examined sunflower and maize that was wither staked or un-staked and therefore free to move and bend in the wind. Free-standing plants developed a wider stem at the base and reductions in the fresh weight of leaves. The bending strength of maize plants significantly increased if the plant was able to move freely. Crook and Ennos (1996) compared free-standing and supported wheat plants. These plants were grown outside with support added at the beginning of stem extension and maintained until the plants were harvested at maturity. They found no difference in height, or ear weight between static and free-standing plants, though the stems of static plants had a significantly lower bending strength and bending rigidity. Prior to the addition of support in these two

experiments, the plants were grown outside in-situ where some would later have support added. This period of exposure could, to some extent, already have had an effect on the plants, hence the overall lack of differences between free-standing and supported plants. As seen in chapter three, plants respond strongly to mechanical stimuli during tillering and even small doses, such as a single brushstroke, significantly reduced plant height. It may be necessary to grow plants indoors prior to treatment in order to prevent exposure that may affect both control and treated plants. Additionally, Crook and Ennos (1996) mentioned that support plants were not held totally static, as plants swayed between 2.5° and 5° deflection. Controls or supported plants grown outside would need to be grown in a way that minimises movement as much as possible in order to be directly comparable.

The force of wind against plant parts causes them to bend and flex. Plants need to maintain an optimal growth position to maximise photosynthesis, and they achieve this through sensing where they are in space using gravity and light. Through proprioception plants are able to readjust their angle to maintain a suitable upright position (Gardiner, Berry, and Moulia 2016). Plants are also able to tolerate moderate forces that cause bending of the stem without breaking. However, if the force of the wind is greater than the strength of the plant or root system, it may overcome plant and cause it to topple or the stem to break. Plants subjected to repeated wind loadings bend but do not break and exhibit morphological features associated with mechanical treatment and Thigmomorphogenesis (Ennos 1997). Wind also causes damage to exposed plant parts. The action of particles carried by the wind can result in abrasion and sandblasting. Moving parts of plants may also collide causing further abrasion, but also damage through taring and stripping leaves (Cleugh, Miller, and Böhm 1998).

In addition to the mechanical component of wind, it causes an increase in airflow around the plant. This increase in airflow affects heat, water vapor and carbon dioxide in and around the plant (Grace 1977). Airflow reduces boundary layers, which can exacerbate water stress as well as increasing transpiration, which results in a reduction in leaf temperature. Increases in transpiration may be minor, but the exacerbation of water stress by wind can significantly affect plant development (Onoda and Anten 2011). Anten et al. (2010) studied the effects of mechanical stimuli and wind on plantain and found that responses to the two could be different, and even in the opposite direction.

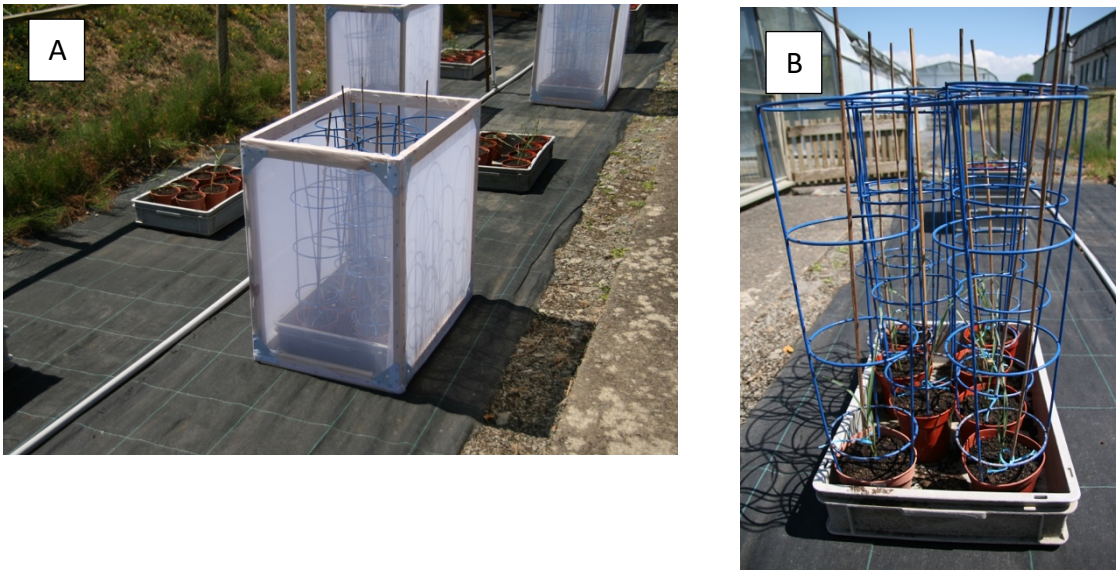
Chapters four and five focused on the study of wheat grown in relatively controlled conditions with airflow that was close to static. Plants were brushed using a rig that moved them forwards and backwards, inducing a consistent bending along one axis. Wind loading also induces a bending moment but is much more dynamic and flexes plants in multiple directions and at different levels of intensity (Gardiner, Berry, and Moulia 2016). Therefore, it would be interesting to see how the wheat plants respond to the natural wind conditions compared with statically grown plants. Does wind provide enough of a force to induce bending and elicit responses similar to those seen in previous experiments? Furthermore, does the application of brushing in addition to wind exposure add to the mechanical response, or is the response already saturated by the winds mechanical effect?

The aim of this chapter is to investigate the response of wheat plants grown outside to natural wind treatment, compared with plants that are prevented from moving and held under static

conditions. Additionally, brushing wind treated plants will be studied to determine if there is an additive effect of this form of mechanical treatment.

6.2 Materials and methods

160 seeds of Mulika (Senova seeds) were planted into 5 inch pots containing John innes number 3 compost, watered daily and left to germinate in a greenhouse with supplementary heating (20°c day/10°c night) and lighting (10 hours day duration). Main tillers were tagged when plants consisted of three tillers using brightly coloured wool thread. Two weeks after planting, plants with poor or excessive growth were removed along with pots containing unterminated seeds. The remaining plants were moved to an outside area of hard standing. These plants were than randomly distributed across nine groups of eight each, and each group was assigned to one of three treatments – static, wind, and brushing. Each group of eight plants assigned to the static treatment was surrounded with a purpose-built baffle to reduce airflow around and between the plants (Figure 71a). Each individual plant within the group



was also staked to a basket-like frame in order to further reduce plant movement and leaf

Figure 71 - (A) Purpose built baffles were created using a fine mosquito netting doubled over and secured to a wooden frame. Windspeed inside the baffle was measured using an Omega instruments handheld anemometer at less than 0.5 m/s. (B) Plants inside the baffles were also secured to frames to reduce movement and leaf flutter.

flutter (Figure 71b). Both the wind treated and brushed plants remained unsupported and thus were able to sway and move under natural wind conditions. Plants receiving brushing

treatment received 20 brushstrokes once per day using the same rig as described in Chapter 2. Plants were watered daily using overhead sprinklers in addition to natural precipitation. At two weeks after seed germination, treatment began, and lasted for four weeks (A photo of the experimental set-up can be seen in Figure 72). At the end of the four weeks of treatment (T1), data on the growth and development of plants was recorded, including plant height to highest point, plant height to base of flag leaf, number of tillers produced by each plant, and flag leaf length and width. Developmental characteristics such as the distance between the flag leaf base and the ligule of the second leaf were measured and a note was made of the dates of anthesis for each plant.

Additional brushing was halted, and plants left to grow under natural conditions until plants reached growth stage 69 – end of flowering, when additional phenotypic measurements were taken (T2). At this point, the number of flowers produced by each plant was recorded, along

Figure 72 - Wheat plants were grown outside on hardstanding. 'Static' plants were surrounded by baffles while all other plants were exposed to natural wind conditions. Brushed plants received 20 brushstrokes once per day and were placed upon boxes during treatment to ensure the rig was in the right position for treatment.

with the height of the main tiller, length and diameter of each internode on the main tiller, and main tiller flag leaf length and width. Four plants from each group (12 from each treatment) were selected for 3-point bending mechanical testing and measurements using the same methods as described in the previous chapter.

Remaining plants were left to grow and mature under natural conditions. Once the plants had become senesced, final phenotypic measurements were taken (T3), including a count of flowering spikes per plant main tiller spike length and the lengths of all additional spikes on each plant. Spikes were then tagged, cut away from the stems and weighed to determine each plant total spike weight. Each main tiller spike was also weighed separately. Remaining plant materiel was cut at the base with a sharp knife and weighed to determine plant biomass, followed by weighing each main tiller stem individually. Main tiller stems were then stripped of leaves and a 50 mm section of the third internode utilised for 3-point bending tests and mechanical measurements. The rest of the material from each plant was separated into leaves and stems, with the leaves being stored in paper bags and the stem material ground using a hammer mill, then stored in polythene sample pots.

Main tiller spikes were sent for CT scanning and the data output was processed as described in previous chapters. The output was grain length, width and count per spike.

Mechanical and grain measurements and statistical processing was conducted as described in the materials and methods section of Chapter 2.

6.3 Results

6.3.1 Main tiller height at the end of treatment

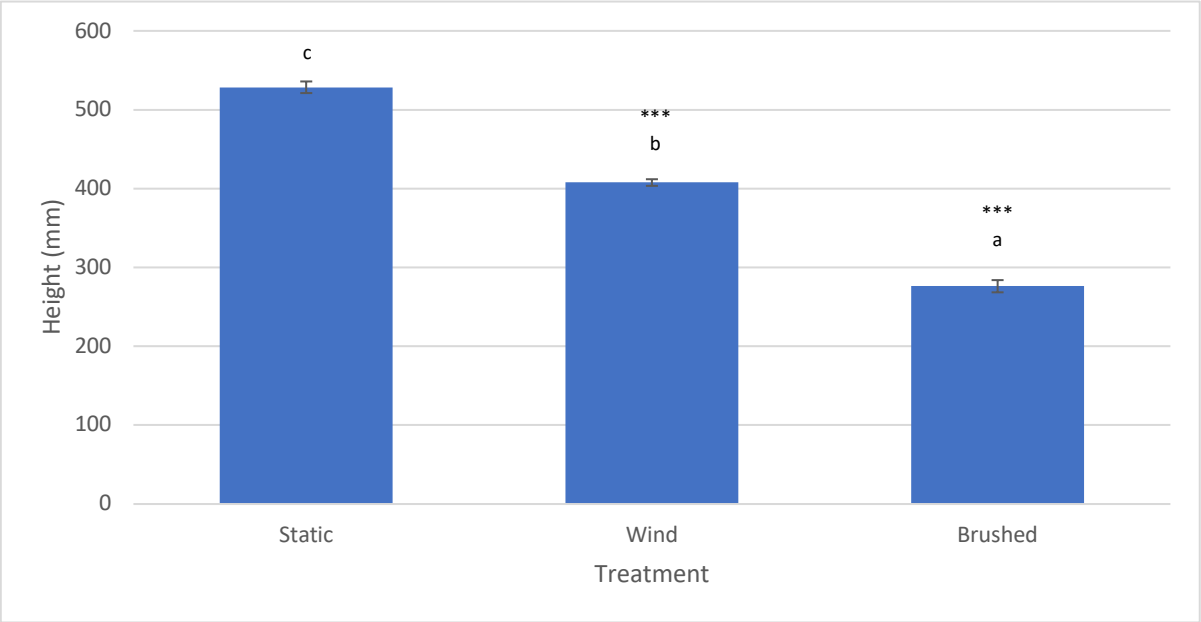


Figure 73 – Main tiller height at the end of treatment (T1). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

The main tiller of wind exposed plants were significantly shorter than statically grown plants (ANOVA $p < 0.001$). On average, static plants were 528.5 mm tall, followed by wind exposed plants (407.7 mm), while brushed plants were the shortest at 276 mm on average (as shown in Figure 73). A Dunnett t post-hoc determined a significant difference between each treatment and static plants – $p < 0.001$.

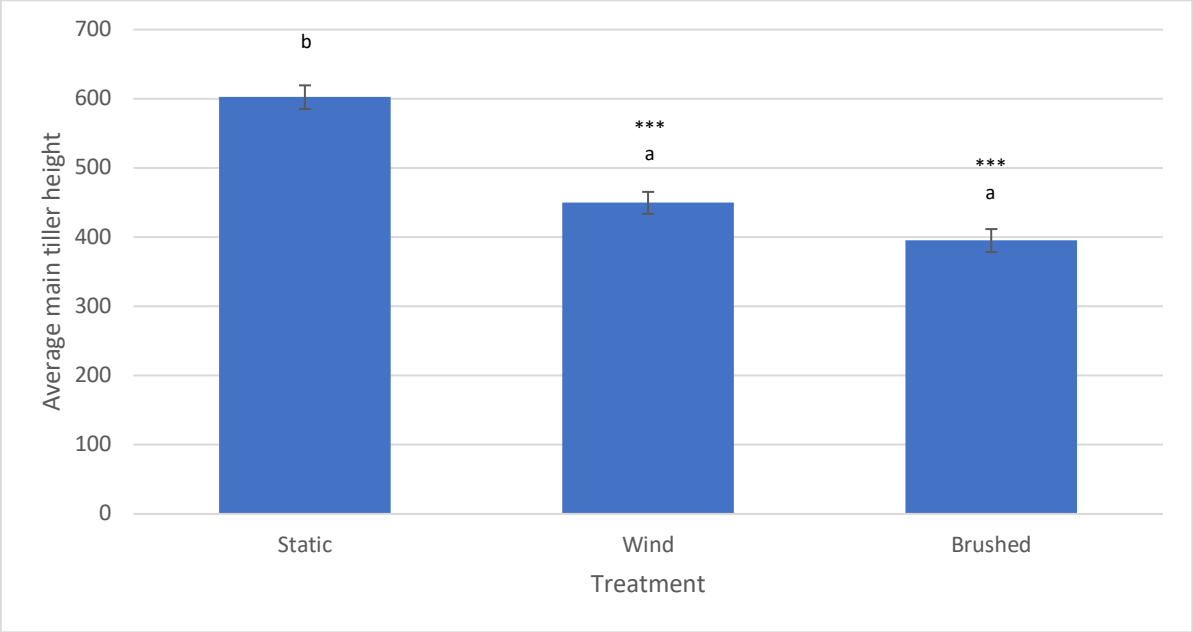


Figure 74 - Mean main tiller height at the end of flowering (T2), measured from soil surface to tip of the flowering spike. Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

When plants had finished flowering (growth stage 69), the height of each main tiller was measured, from the soil surface to the tip of the flowering spike. As seen at the end of treatment, both wind and brushed plants were significantly shorter than static plants (ANOVA $p < 0.001$). Static plants were 602.2 mm tall while wind treated plants were 152.67 mm shorter and brushed plants were 207.19 mm tall on average (Figure 74).

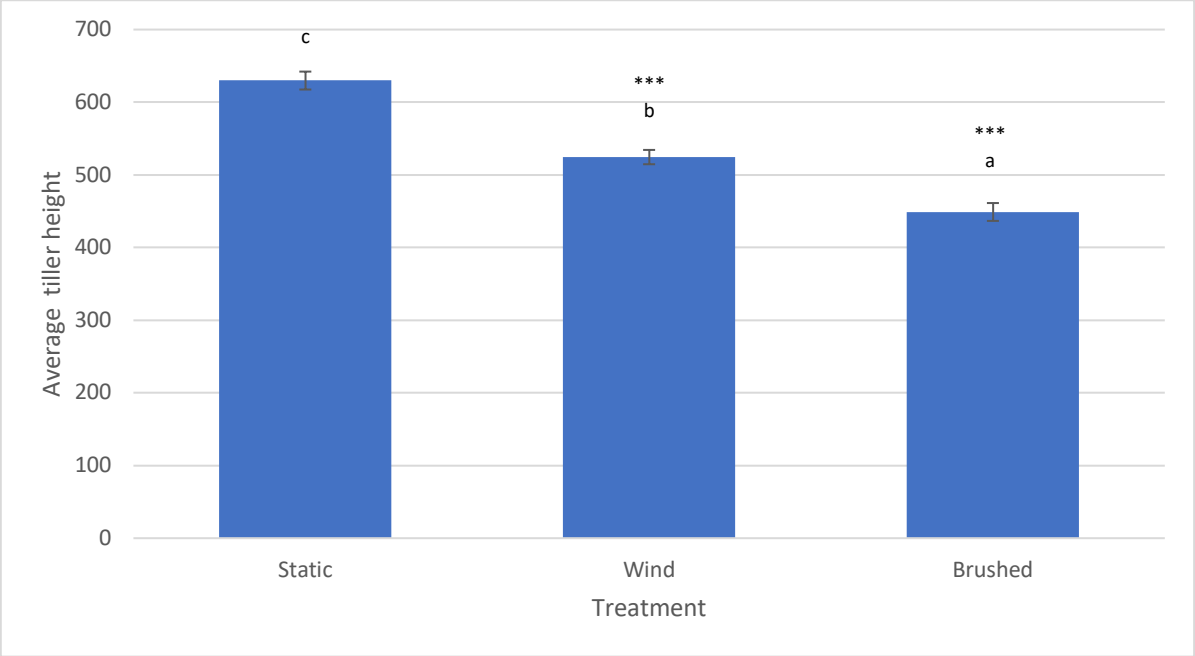


Figure 75 - Mean height across all tillers on each plant at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

At the end of flowering, the height of all tillers on each plant was measured in addition to main tiller measurements, an average tiller height for each plant was then calculated. Plants that received treatment were significantly shorter than those grown in static conditions (ANOVA $p < 0.001$). Static plants had an average tiller length of 629.8 mm, while wind plants were 524.5 mm and brushed were 449.0 mm tall (Figure 75). There was also a significant difference in tiller height between wind and brushing treatments (Tukey HSD $p<0.001$).

6.3.2 Internode length

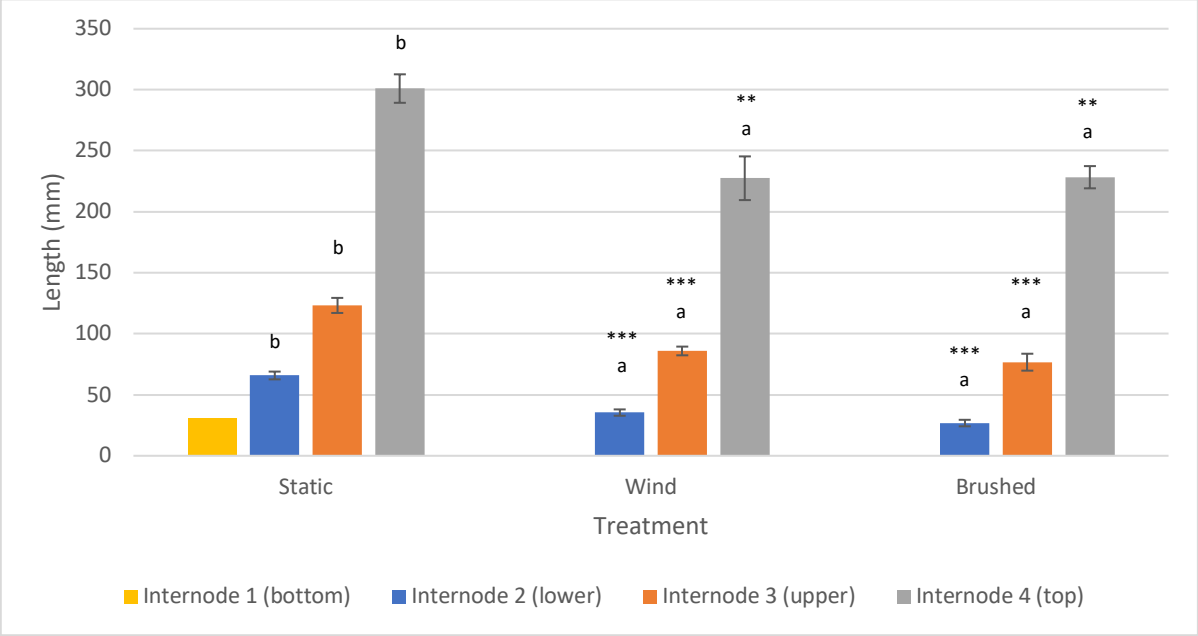


Figure 76 - Length of internodes from 1 (bottom) to 4 (top) at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

After measuring the main tiller height, the length of each individual internode on the main tiller was measured from the bottom to the top and numbered 1 to 4. Of the treated plants, only one wind treated plant produced four internodes. Only 11 of the statically grown plants produced 4 internodes, which were 30.3 mm long on average. The second internode of statically grown plants was 65.8 mm long on average, significantly longer than the second internode of wind treated plants (35.4 mm) and brushed plants (26.8 mm, ANOVA $p<0.001$.) The pattern was again repeated for internode 3, with plants that were free to sway in the wind, including those that received additional brushing, producing significantly shorter third internodes than static plants ($p < 0.001$). The top internode of treated plants was also significantly shorter than for static plants (ANOVA $p < 0.001$). There was no significant difference I the length of internode 4 between the two treatments, shown in Figure 76.

One of the main causes of the difference in height between static and treated plants was due to the lack of base internode (internode 1). Additionally, treatment reduced the length of all three remaining internodes, resulting in an overall reduction in the height of main tillers compared with static plants.

6.3.3 Internode diameter

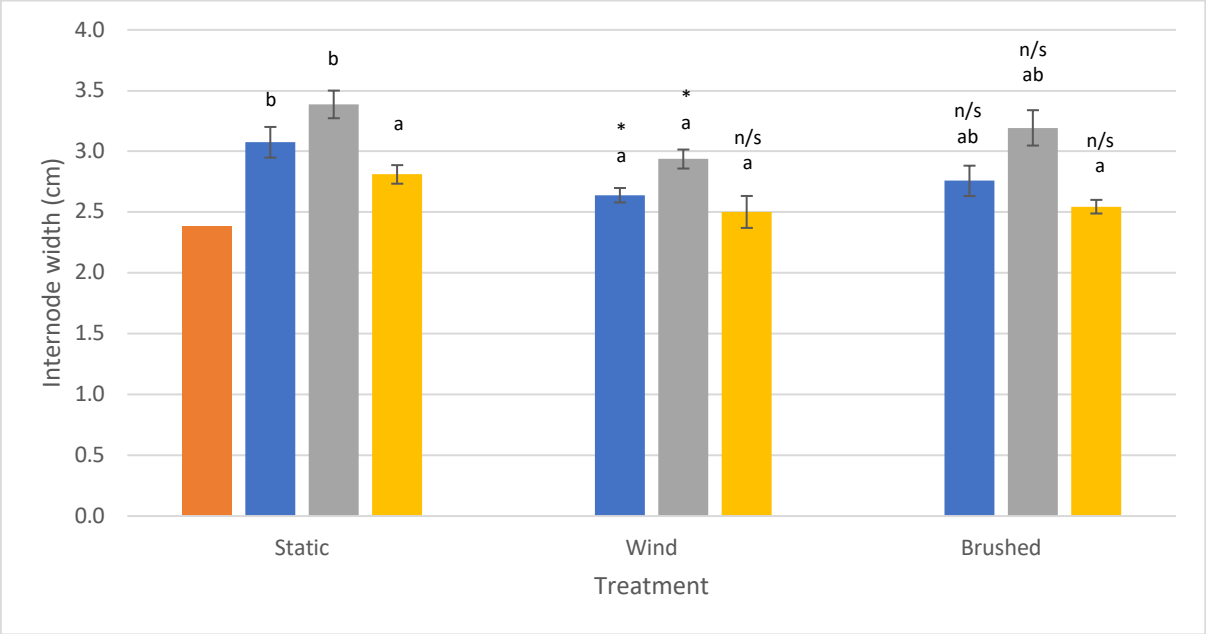


Figure 77 - Diameter of internodes 1 (bottom) to 4 (top) at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

At the end of flowering the diameter of each internode on the main tiller was recorded. Only static plants consisted of four full internodes, and the average diameter of the first internode of static plants was 2.39 mm. The average diameters for all other internodes can be seen in Figure 77. Wind treated plants had a narrower second and third internode than both static and brushed plants, both of which were significantly narrower than for static plants ($p<0.05$), but there was no significant difference between wind and brushing treatments. The fourth internode was unaffected by treatment.

6.3.4 Tillers and flowers

6.3.4.1 Tiller count

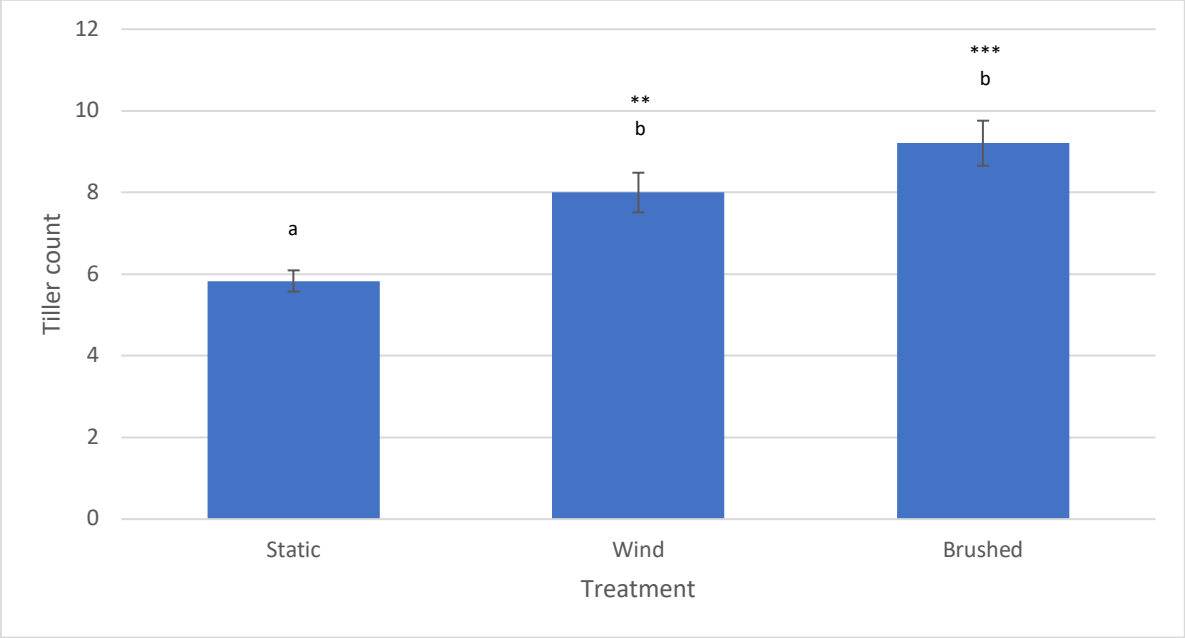


Figure 78 - Mean number of tillers per plant at the end of treatment (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

As seen in previous experimental chapters, treated plants had more tillers than static plants, which had an average of 8.8 tillers per plant (Figure 78). Wind plants had 8.0 and brushed plants an average tiller count of 9.2, with both treatments having a significantly greater number of tillers than static plants (ANOVA $p<0.001$). There was no significant difference in tiller numbers between the two treatments.

6.3.4.2 Flowering spike count

There was little difference in the number of flowering spikes per plant when recorded at the end of flowering, and an analysis of variance indicated there was no significant difference in spike numbers.

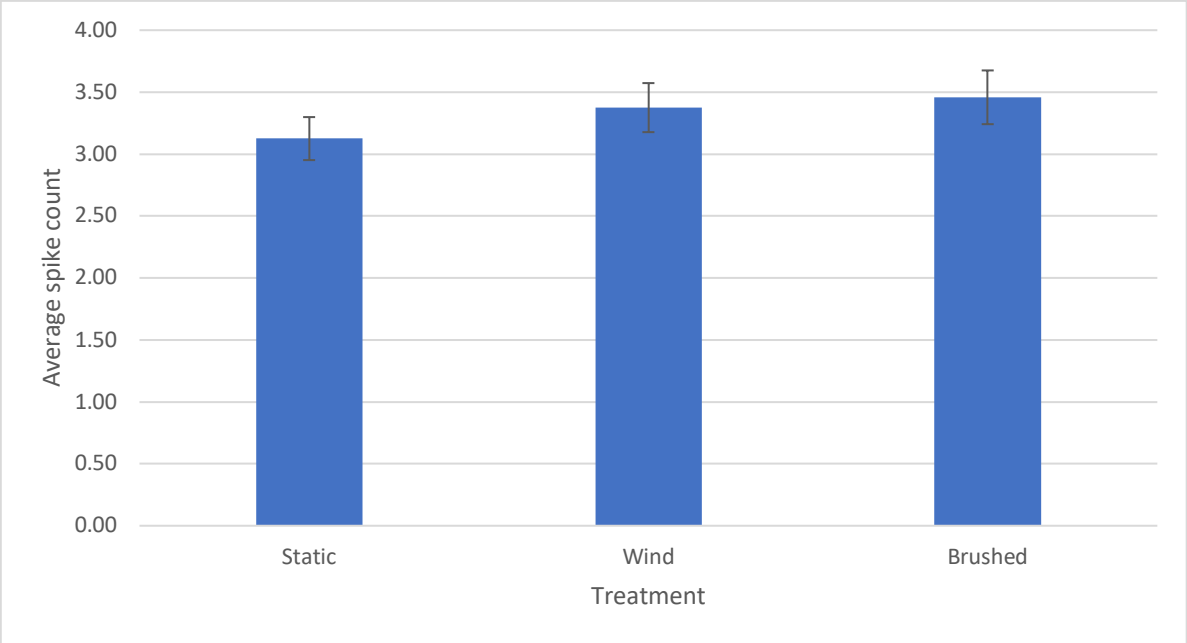


Figure 79 - Mean number of flowering spikes per plant at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. n=24.

The number of flowering spikes per plant was counted again at maturity to see if there were any tiller death between flowering and maturity, and to check for spike fertility (Average spike count shown in Figure 79). There was no difference in the numbers of spike numbers counter at T2 and T3. Therefore, there was no difference in the number of spikes between treated plants and static plants.

Table 9 - Spike measurements from harvested mature spikes (T3). Stars indicates the data is significantly different to controls according to a Dunnett t test. Measurements with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$).

Treatment	Static	Wind	Brushed
MT spike length (mm) n=20			
Mean	75.3	69.1	71.9
s.d.	7.80	8.05	9.56
Sig		n/s	n/s
Plant Mean spike length (mm) n=12			
Mean	79.7	73.9	76.2
s.d.	4.76	5.49	7.29
Sig		n/s	n/s
MT spike weight (g) n=12			
Mean	1.29	1.12	1.14
s.d.	0.587	0.568	0.712
Sig		n/s	n/s
Total spike weight per plant (g) n=12			
Mean	5.91	5.56	5.63
s.d.	1.878	1.903	2.166
Sig		n/s	n/s
Spike count n=12			
Mean	3.13	3.46	3.38
s.d.	0.850	1.062	0.970
Sig		n/s	n/s
Plant average spike weight (g) n=12			
Mean	1.72	1.43	1.70
s.d.	0.32	0.167	0.324
Sig		*	n/s
	b	a	ab

Static plants had an average main tiller spike length of 75.3 mm, while wind treated plants were 71.9 mm and the main tiller spikes of brushed plants were 69.2 mm long on average. An analysis of variance did not identify any significant difference in the length of spikes between treated and static plants.

There was no significant difference in the weight of individual main tiller spikes between treated and static grown plants (Table 9). Statically grown plants had an average total spike weight of 5.91g, while wind treated plants were 5.63g and brushed plants were 5.56g on average. There was no significant difference in the total spike weight per plant between treated and static plants or between the treatments. When the total weight of spikes from each plant was divides by the number of spikes the plant yielded, static grown plants had an average spike weight of 1.72g, wind treated plat spikes weighed 1.70g and brushed were 1.43g. There was no significant difference in the average spike weight between static and treated plants, however, there was a significant difference in average spike weight between the two treatments (ANOVA Tukey HSD post hoc $p<0.05$).

6.3.5 Developmental traits

Both wind and brushing treatments resulted in a shorter length form soil to base of flag leaf compared with statically grown plants (Table 10). An analysis of variance indicated that treated plants were significantly difference to static plants – $p<0.001$, and a Tukey HSD post hoc showed a significant difference between the two treatments.

At the end of treatment, all plants were measures on the same day to give a rough estimation of developmental differences between treated and static plants. The distance between the main tiller auricle and ligule of the second leaf can be used to identify differences in developmental stages between plants. Static plants had an average length of 167.8 mm, while plants that received treatment had a significantly shorter length – 132.1 mm for wind and 96.6 mm for brushed plants ($p<0.001$). A Tukey HSD post-hoc identified a significant difference in auricle to ligule length between the two treatments, $p<0.001$.

The distance between soil surface and ligule was measured again at the end of flowering. Again, there was a significant reduction in the height to flag leaf of treated plants (ANOVA $p<0.001$), however there was no significant difference between treatments this time.

At the end of flowering, the distance between the flag leaf and the bottom of the spike was measured on each main tiller. There was a significant difference in length from flag leaf base to spike base for treated plants $p<0.01$, though the difference was less than for equivalent measurements (auricle to ligule) taken at the end of treatment. There was no significant difference in length between the two treatments.

Table 10 - Plant developmental trait measurements. Recorded at the end of treatment (T1) and the end of flowering (T2). Stars indicates the data is significantly different to controls according to a Dunnett t test. Measurements with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$).

	Treatment		
	Static	Wind	Brushing
T1 - End of treatment			
Height to flag leaf n=24			
Mean	398.3	292.5	220.7
s.d.	48.13	29.00	18.98
Sig		***	***
	c	b	a
Auricle to Ligule n=24			
Mean	167.8	132.1	96.6
s.d.	22.82	18.13	17.69
Sig		***	***
	c	b	a
Flag leaf length n=24			
Mean	349.0	308.1	264.0
s.d.	38.00	30.03	29.49
Sig		***	***
	c	b	a
T2 - End of flowering			
Height to base of flag leaf n=24			
Mean	409.9	302.3	271.9
s.d.	52.85	38.20	65.43
Sig		***	***
	b	a	a
Base of flag leaf to base of spike n=24			
Mean	123.5	78.2	69.2
s.d.	46.96	56.84	39.47
Sig		**	**
	b	a	a
Flag leaf length n=24			
Mean	353.2	278.0	225.8
s.d.	57.19	23.34	33.92
Sig		***	***
	c	b	a

6.3.6 Flag leaf measurements

Flag leaf length was initially measured at the end of treatment. Wind treated and brushing treated plants had significantly shorter flag leaves than static plants, ANOVA $p<0.001$ (see table 10). Static plants had an average flag leaf length of 349.0 mm, while wind treated plants were 40.9 mm shorter and the leaves of brushed plants were 85.0 mm shorter. The difference in flag leaf length between wind and brushing treatments was also identified as statistically significant (Tukey HSD $p<0.001$).

Flag leaf area was calculated using the measured flag leaf length and width multiplied by a conversion factor of 0.74. The main tiller flag leaf of static grown plants was 4134.3 mm^2 on average, whereas treated plants had smaller flag leaf areas – 3620.8 mm^2 for wind treated and 1423.9 mm^2 for brushed plants (Figure 80). There was no significant difference in flag leaf area between statically grown plants and wind treated plants, however, brushed plants were significantly less than static grown plants ($p<0.001$).

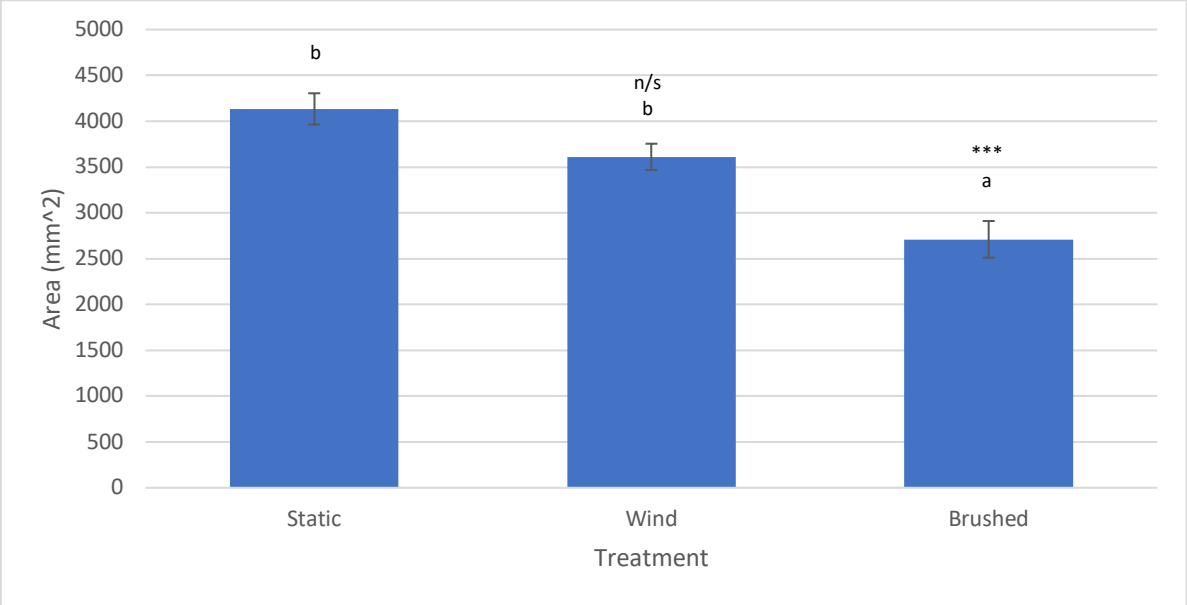


Figure 80 - Main tiller flag leaf area at the end of treatment (T1). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

Main tiller flag leaf measurements were repeated again at the end of flowering (see Table 10), and again there was found a significant difference between static and treated plants (Dunnett t p <0.001) and also between the two treatments (Tukey HSD p<0.001).

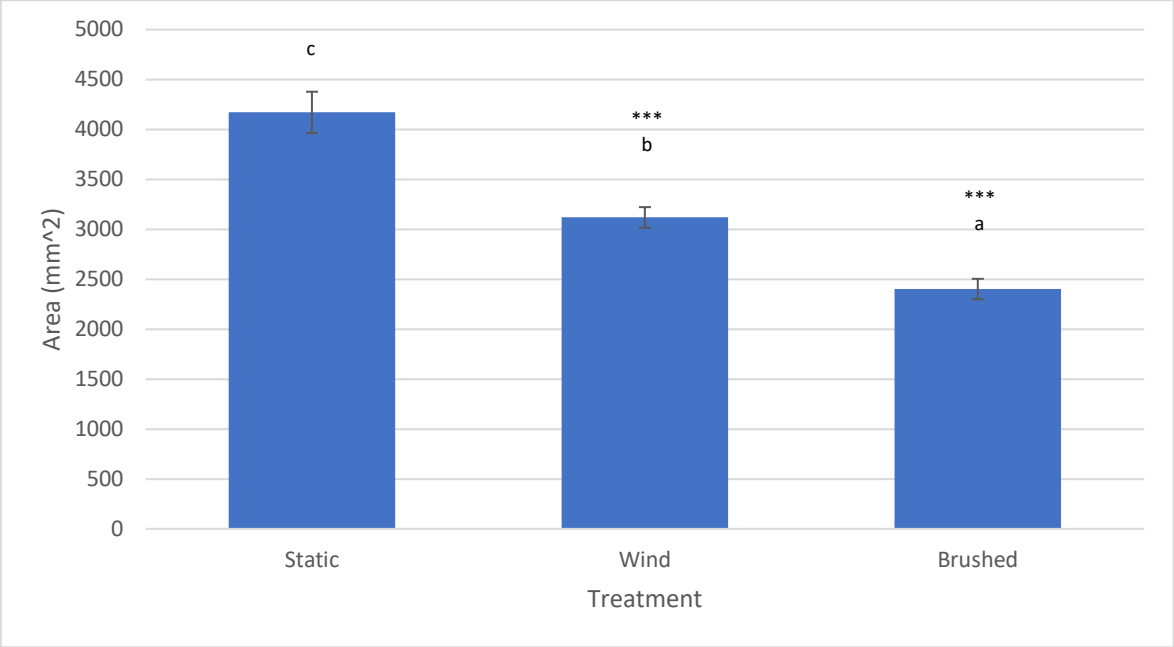


Figure 81 - Main tiller flag leaf area at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=24.

The area of main tiller flag leaves measured at the end of flowering was also found to be significantly different between static and treated plants (ANOVA $p<0.001$) and also between wind treated and brushing treated plants (Tukey HSD $p<0.01$). As shown in Figure 81, static plants had a much larger main tiller flag leaf area (4170.5 mm^2) than either wind treated (3118.3 mm^2) or brushing treated plants (2401.6 mm^2).

6.3.7 Biomass (excluding spikes)

6.3.7.1 Main tiller

The stems of plants that received mechanical treatment weighed less than plants grown in static conditions, though only the plants which received the brushing treatment were significantly different in main tiller stem weight to static plants (ANOVA $p<0.01$). The main tiller of static plants weighed 1.30g on average, while wind treated plants weighed 0.25g less, and brushed plants weighed 0.55g on average (Figure 82).

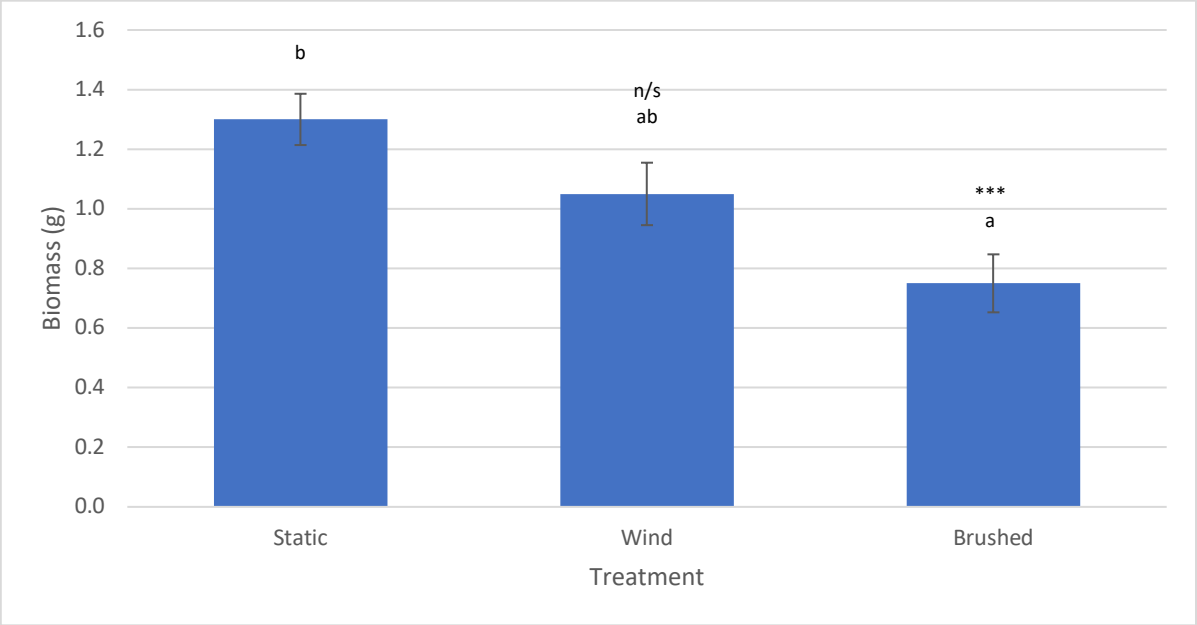


Figure 82 - Biomass of the Senesced mature main tiller stem (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

6.3.7.2 Whole plant biomass

Statically grown plants had the greatest biomass at maturity, 5.40g per plant, compared with wind treated, 4.48g and brushed plants, which weighed 4.14g on average (Figure 83). Treatment did not have a significant effect on plant biomass.

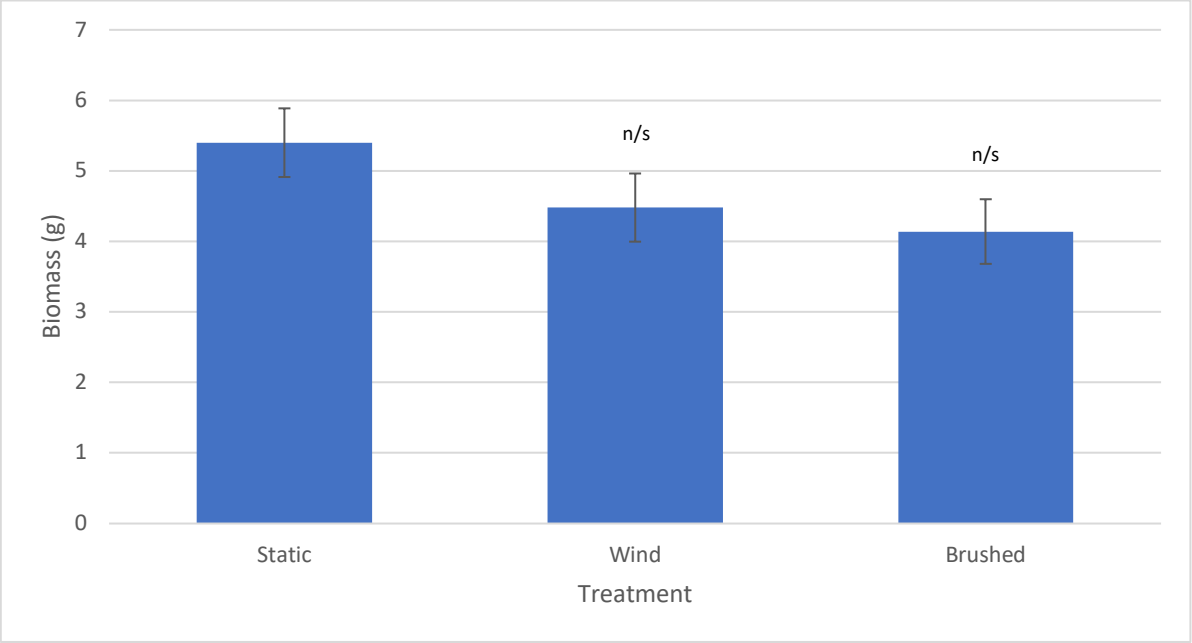


Figure 83 - Biomass of senesced mature wheat plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

6.3.8 Mechanical properties of stems at the end of flowering

6.3.8.1 Stem measurements

Cross-sections were photographed, and the images used to measure outer and inner stem diameters, which were then used to calculate the area of the cross-section. At T2, wind treated plants had the narrowest third internode (3.07 mm) followed by brushed plants (3.37 mm), while static plants had an average internode 3 diameter od 3.47mm (Table 11). Only wind treatment had a significant effect on the outer diameter of internode 3 (Dunnett t $p<0.05$). Wind treatment had a significant effect on inner stem diameter, with inner stem diameters averaging 1.15 mm less than statically grown plants (Inner diameter of 1.36 mm), Dunnett t $p<0.05$. Plants that received brushing treatment were 0.22 mm larger internally than statically grown plants, though the difference was not significant.

Table 11 - Stem measurements at the end of flowering and a maturity when plants had senesced. Stars indicates the data is significantly different to controls according to a Dunnett t test.

Treatment		Static	Wind	Brushed
Stem diameter at the end of flowering, T2 (mm)				
Outer	Mean	3.47	3.07	3.37
	s.d.	0.370	0.260	0.557
	Sig		*	n/s
Inner	Mean	1.36	0.21	1.59
	s.d.	0.463	0.068	0.816
	Sig		*	n/s
Stem diameter at maturity, T3 (mm)				
Outer	Mean	3.56	3.14	3.08
	s.d.	0.343	0.308	0.548
	Sig		*	*
Inner	Mean	1.70	1.16	1.29
	s.d.	0.386	0.166	0.554
	Sig		*	n/s
Number of hollow stems		7	3	6
Number of pithy stems		5	9	5
N		12	12	11

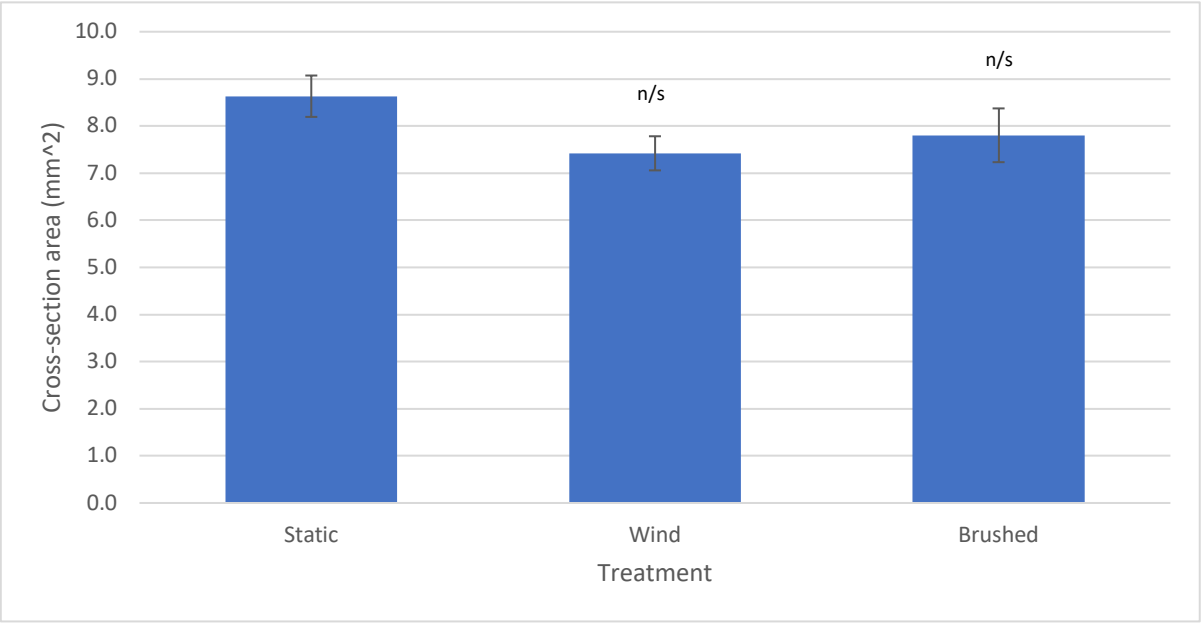


Figure 84 - Main tiller internode 3 cross-section area at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Both treatments resulted in a reduced cross-section area compared with static grown plants, though an analysis of variance did not identify the difference as being significant. Static grown plants had an average internode 3 cross-section area of 8.63 mm^2, while brushed plants measured 7.80 mm^2 and wind treated plants 7.42 mm^2 on average (Figure 84).

6.3.8.2 Bending strength

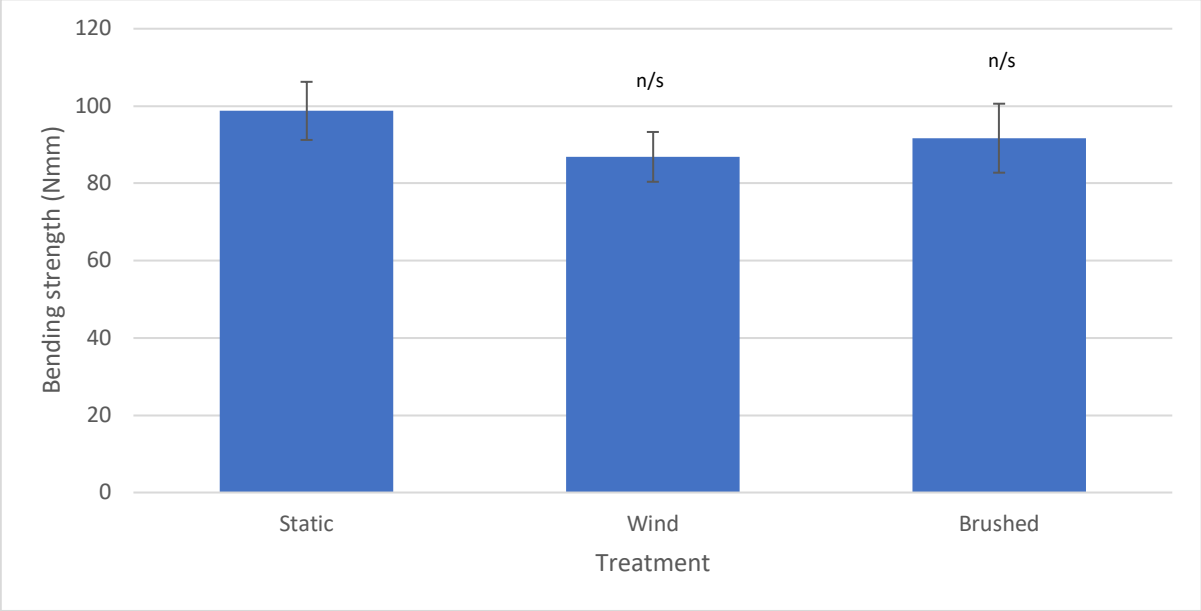


Figure 85 - Main tiller internode 3 bending strength at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Wind treated plants had a lower bending strength (86.84 Nmm) than both brushed plants (91.44 Nmm) and static plants (98.74 Nmm), though neither treatments had a significant effect on the bending strength of the third internode (Figure 85).

6.3.8.3 Bending rigidity

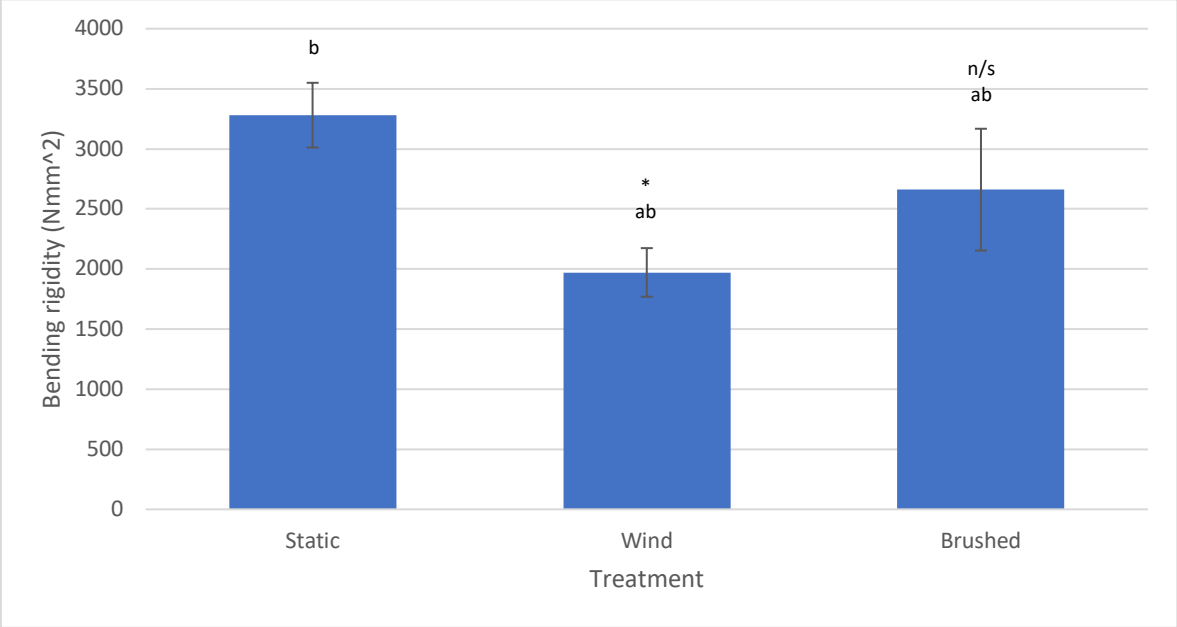


Figure 86 - Main tiller internode 3 bending rigidity at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Again, wind treated plants had a lower bending rigidity than either brushed plants or statically grown plants. The average bending rigidity of static plants was 3280.5 Nmm^2, compared with 2660.7 Nmm^2 for brushed plants and 1971.6 Nmm^2 for wind treated plants (Figure 86). Only the wind treatment had a significant effect on the bending rigidity of the third internode of main tillers, Dunnett t $p<0.05$.

6.3.8.4 Young's Modulus

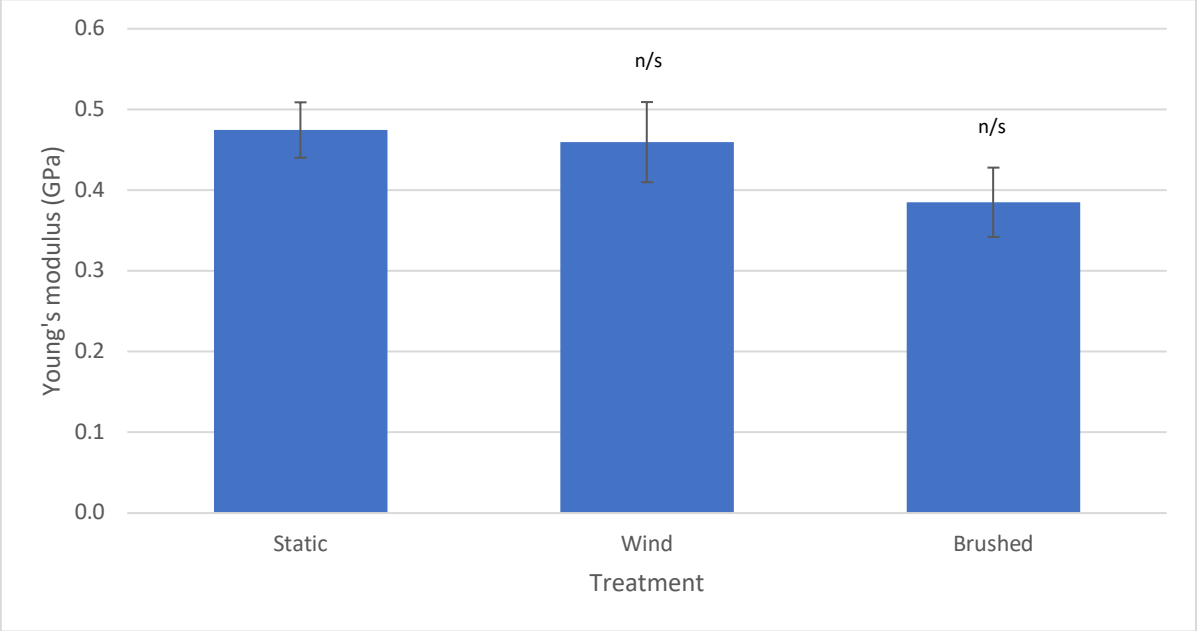


Figure 87 - Main tiller internode 3 Young's modulus at the end of flowering (T2). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Treated plants had a lower Young's modulus than static plants, however the trend as seen in bending strength and rigidity did not continue. Brushed plants had the lowest young's modulus (0.385 GPa) followed by wind (0.460 GPa), and then static (0.475 GPa). Data shown in Figure 87) An analysis of variance test indicated that nether treatments had a significant effect on the Young's modulus of the third internode of plants harvested at the end of flowering.

6.3.9 Mechanical properties of mature stems

6.3.9.1 Outer stem diameter

After mechanical tests were conducted, the internode sections were cut into thin sections with a sharp razor blade and photographed under a microscope at x10 magnification. Images were measured on the computer for inner and outer stem diameter, which were then used to calculate the cross-section area. Data shown in table 11. Plants that received treatment had significantly thinner stems than statically grown plants (ANOVA, $p<0.05$). Static plants had an average outer diameter of 3.56 mm, while wind treated plants were 3.14 mm wide and internode sections of brushed plants were 3.09 mm wide.

6.3.9.2 Inner stem diameter

Wind treated plants had significantly smaller inner diameters than statically grown plants (0.54 mm difference, ANOVA $p<0.05$). Brushed plants also had a narrower internal diameter than statically grown plants (0.41 mm), though not significantly different (Table 11).

6.3.9.3 Cross-section area

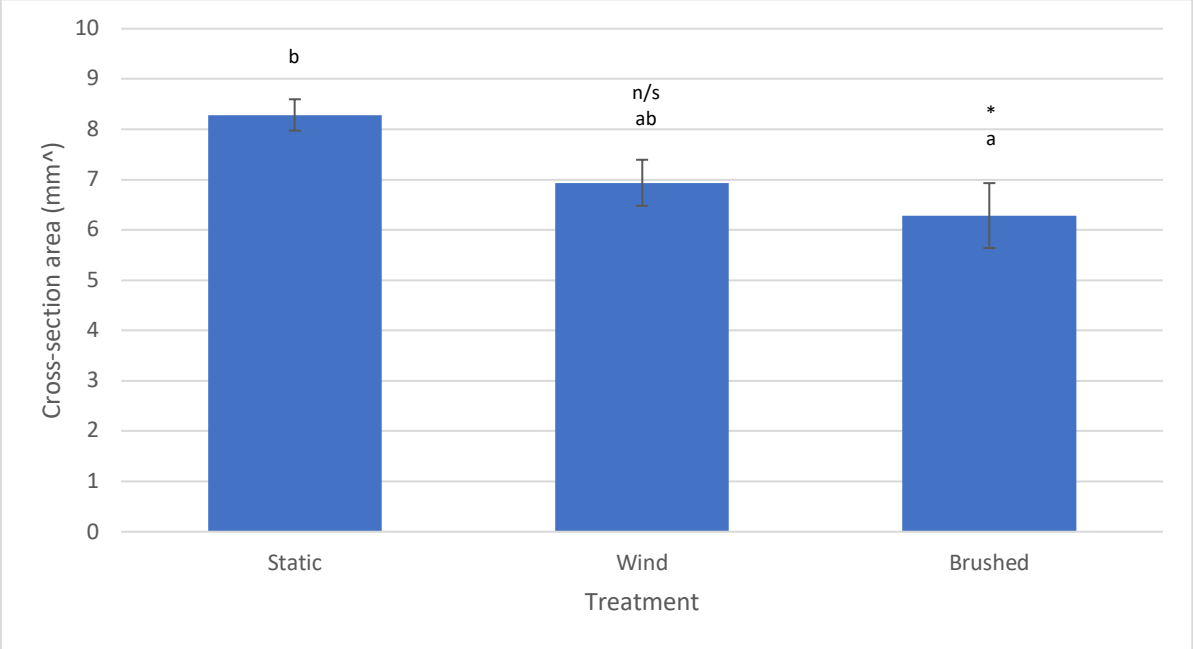


Figure 88 - Cross section area of internode 3 from mature senesced plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

When the cross-section area of each main tiller third internode was calculated, brushed plants had the lowest cross-section area (6.29 mm^2) followed by wind treated plants (6.94 mm^2) then static (8.29 mm^2), shown in Figure 88. Only the brushing treatment had a significant effect on cross-section area, compared with static plants, Dunnett t $p<0.05$.

6.3.9.4 Bending strength

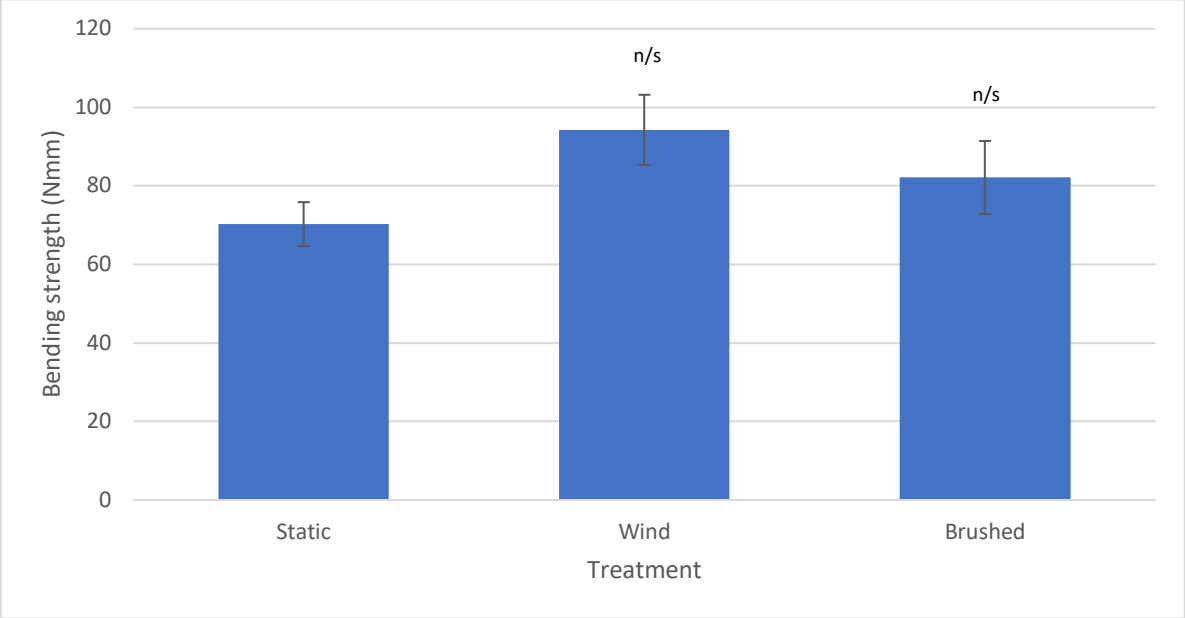


Figure 89 - Bending strength of internode 3 from mature senesced plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

When senesced, treated plants had a higher bending strength than static plants, with wind treated plants having the highest bending strength (94.25 Nmm) followed by brushing treatment (82.09 Nmm) compared with statically grown plants (70.24 Nmm). The difference in bending strength between treated and static plants was not significant (Figure 89).

6.3.9.5 Bending rigidity

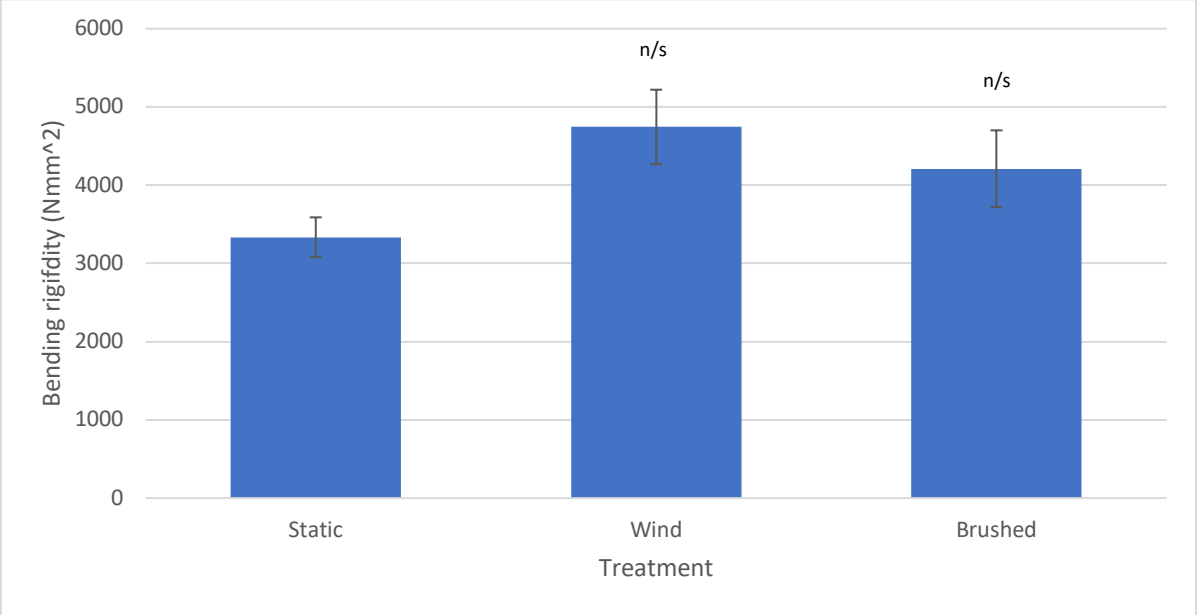


Figure 90 - Bending rigidity of internode 3 from mature senesced plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Wind treated plants had a higher bending rigidity than either brushed or static plants, though the difference was not great enough to be statistically significant (Figure 90).

6.3.9.6 Young's Modulus

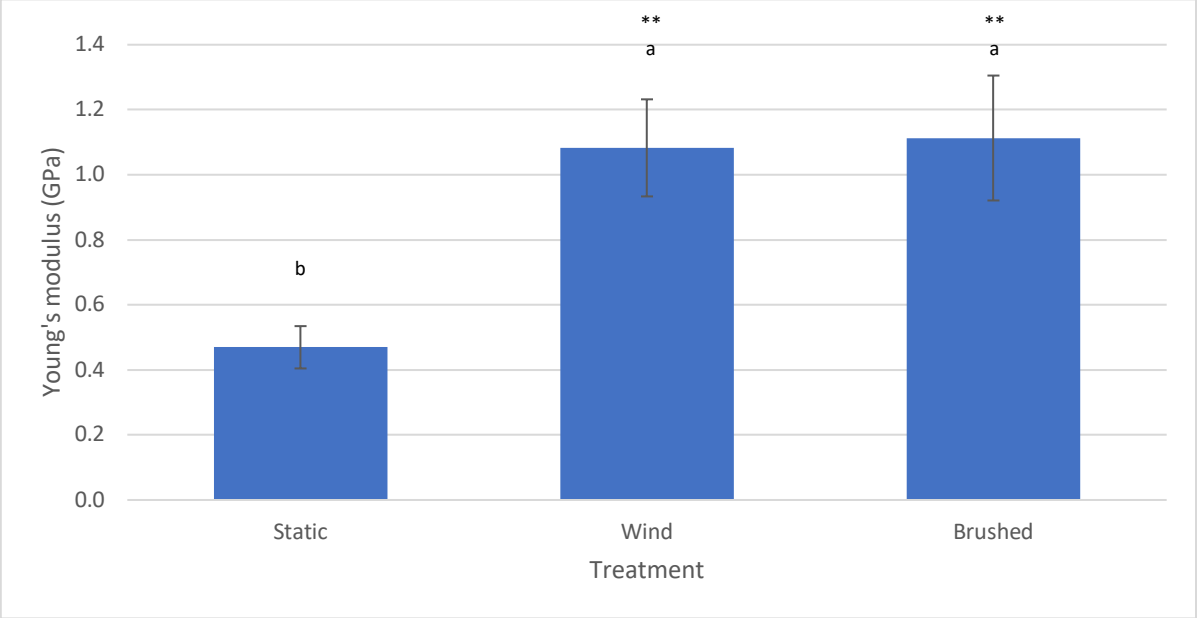


Figure 91 - Young's modulus of internode 3 from mature senesced plants (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Treated plants had a significantly greater Young's modulus than statically grown plants (ANOVA $p<0.01$). A post-hoc Dunnett t test determined that the difference between static and brushed plants ($p<0.01$), which had an average Young's modulus of 1.13 GPa, was greater than with wind treated plants ($p<0.05$), where the Young's modulus was 1.083 GPa (Figure 91).

6.3.10 Grain and yield measurements

6.3.10.1 Grain length and width

Grain length and width were analysed to give an idea of the shape of grains produced by treated and static plants. The grains of static plants were 4.61 mm long on average, longer than either wind treated (4.51 mm) or brushed plants (4.35 mm) (Table 12). Brushing treatment resulted in significantly shorter grains compared with static plants (Dunnett t $p<0.001$) and wind treated plants (Tukey $p<0.01$). Likewise, brushing treatment had the greatest effect on grain width, and thus were significantly narrower that both static plants (3.58 mm wide on average), and brushed plants (3.52 mm wide) $p<0.001$. However, the grains of wind treated plants were neither significantly longer nor wider than the grains of static plants.

Table 12 - Grain dimensions, length and width, from harvested main tillers (T3). Stars indicates the data is significantly different to controls according to a Dunnett t test.

	Static	Wind	Brushed
Grain length (mm)			
Mean	4.60	4.51	4.35
s.d.	0.491	0.453	0.480
Sig	b	n/s b	*** a
Grain width (mm)			
Mean	3.58	3.52	3.37
s.d.	0.376	0.400	0.377
Sig	b	n/s b	*** a

6.3.10.2 Grain volume

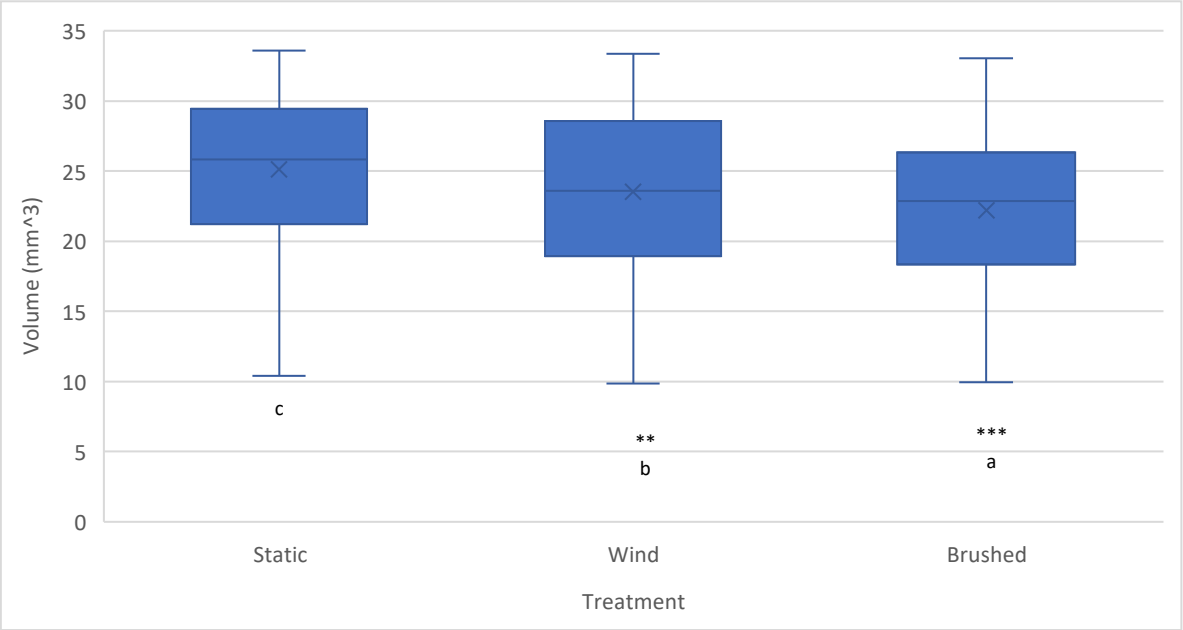


Figure 92 - Individual grain volume of main tillers at T3. In this chart, the upper whisker indicates the top 25% of data, excluding outliers, and the lower whisker indicates the lowest 25% of data, excluding outliers which are marked as dots beyond the whiskers. The X indicates the sample mean and the median value is indicated by the line inside the box. The box itself indicates the interquartile range, where 75% of measurements fall.

Static plants had an average grain volume of 25.12 mm, significantly greater than either wind treated plants, 23.54 mm $p<0.01$, or brushed plants 22.21 mm $p<0.001$. There was also a small but significant difference in grain volume between the two treatments, $p<0.05$ (Figure 92).

6.3.10.3 Grain count

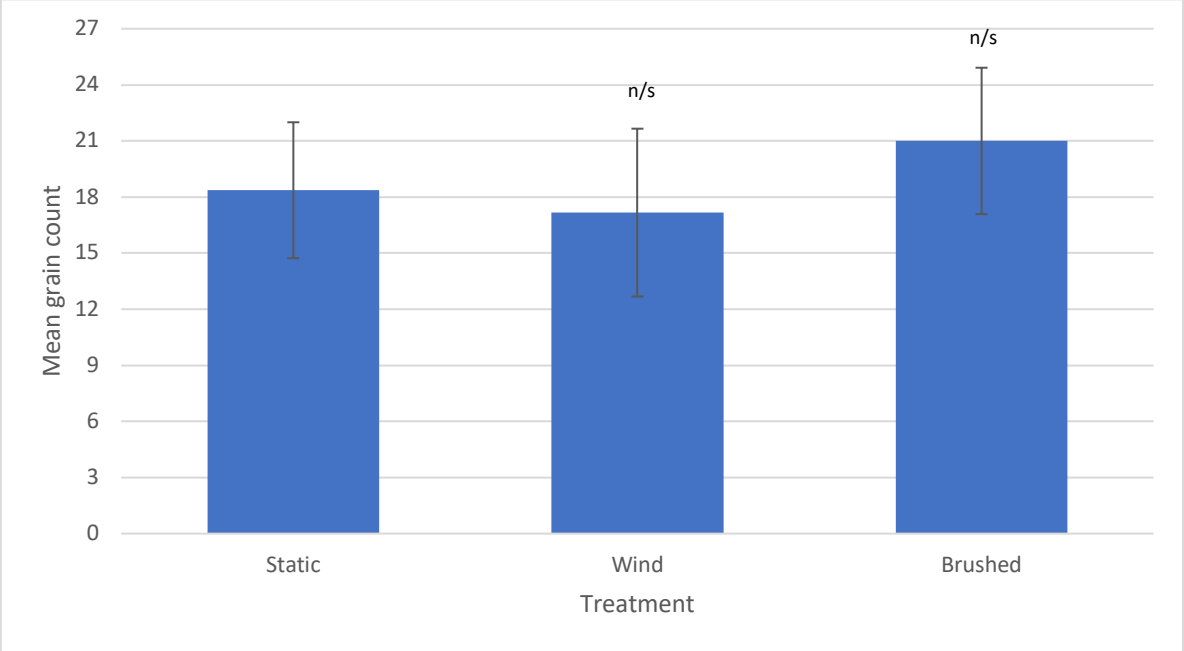


Figure 93 - Mean number of grains per main tiller spike (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Static plants had an average main tiller grain count of 18.4, whereas wind treated plants had fewer grains, 17.2 on average, and brushed plants had more grains per main tiller spike, 21 on average (Figure 93). Differences in grain numbers between treated and static plants were not found to be significant.

6.3.10.4 Total grain volume

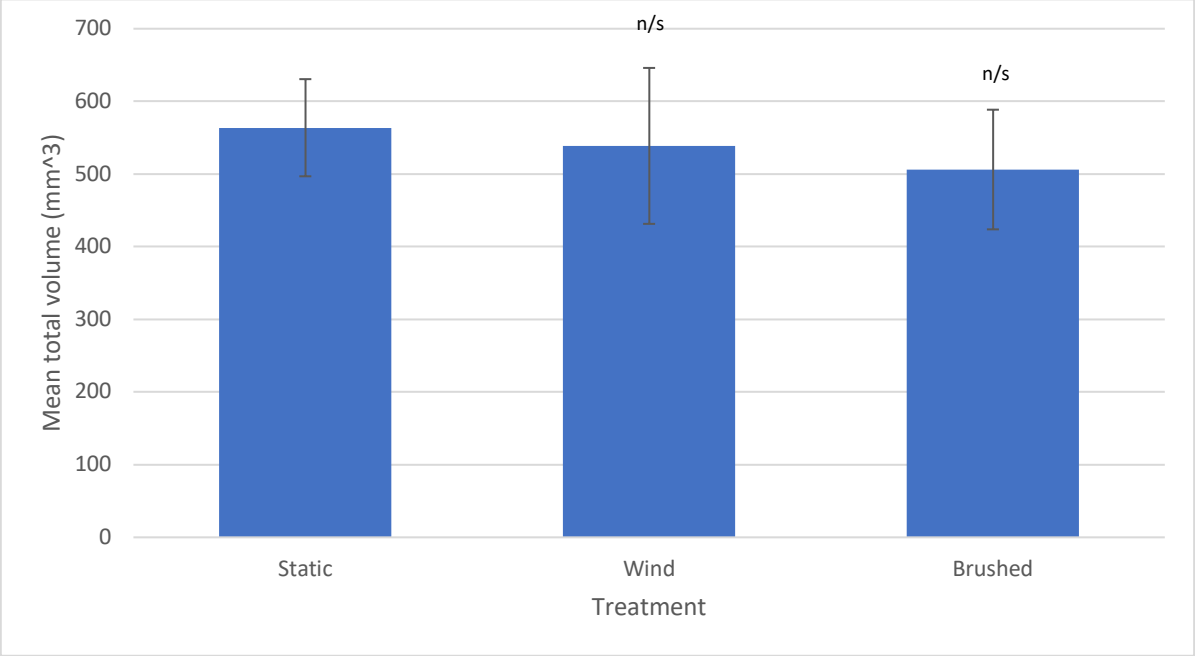


Figure 94 - Total volume of all grains per main tiller spike (T3). Error bars indicate ±1 standard error of the mean. Stars indicates the data is significantly different to controls according to a Dunnett t test. Bars with the same letters are not significantly different according to a Tukeys test($\propto = 0.05$) n=12.

Despite the significant reduction in individual grain volume of both wind treated and brushed plants, there was no significant difference in total grain volume per main tiller. Reductions in individual grain volume were offset by an increase in the number of grains per main tiller for both wind and brushed plants (Figure 94).

6.4 Discussion

6.4.1 Height

There was a significant reduction in the height of main tillers of both wind and brushed plants at the end of treatment. This effect could still be clearly seen at the end of flowering. Both treatments resulted in plants with only three measurable internodes as the first (bottom) internode was either too small to measure or missing completely. Treatment also reduced the length of the second, third and fourth internode, but there was no significant difference in either height or individual internode lengths between wind or brushing treatments.

These results are consistent with those seen in previous chapters, with both wind treatment and additional brushing reducing tiller height. Reductions in the lengths of all internodes suggests that natural wind conditions may be inducing a wider range of bending movements or motions in the plant than brushing alone.

Crook and Ennos (1996) found no difference in stem height between supported and free-standing wheat plants, whereas Goodman and Ennos (1997) found that supported maize plants were 11% taller than free-standing plants. Though the difference between the two experiments is likely to be because the supported wheat plants may still have been able to move to some extent.

There was a very noticeable pattern in the response of internodes to treatment, which indicates that response to mechanical stimuli can be very localised. Treatment resulted in such a significant reduction in the lowest internode that either it could not be measured or

was not present at all. Lower sections of the plant are likely to be exposed to the highest amounts of mechanical stress, as this is where the bending moment is greatest (Goodman and Ennos 1997).

The supports and wind baffles used in this experiment appear to be very successful in reducing wind induced plant movement, and therefore clear differences between supported and free-standing plants can be seen.

6.4.2 Tillers and spikes.

Wind treatment resulted in significantly more tillers than static plants, with additional brushing resulting in an even greater tiller number. However, by the end of flowering differences between treatment had evened out which resulted in no significant differences in spike numbers per plant. An increase in tillering was also seen in the experiments in chapters 4 and 5, but these were accompanied with an increase in the number of flowers, whereas the treated plants in this experiment did not have significantly more. This is most likely due to an increase in tiller death due to less-favourable or variable environmental conditions. Temperatures outdoors were much less stable in the greenhouse, and despite additional irrigation, plants may have experienced moisture or heat stress during particularly hot and dry periods.

As soon as it elongated starts, tillers begin to die off with the most recently produced dying first. Environmental conditions such as solar radiation, temperature, nutrient stress, and drought/moisture stress can affect both the number of tillers produced but also the number lost (Acevedo, Silva, and Silva 2002, Thorne and Wood 1987, Yang et al. 2019). Jaffe (1993)

noted that other studies reported a decrease in the number of flowers and subsequently fruits and seeds in various species in response to mechanical treatment. He suggests that this may be an advantage, allowing the plant to ensure successful reproduction by reducing the chance of damage due to mechanical stress or high winds.

The lack of differences flower numbers was reflected in the whole plant biomass, as there was no difference between static and treated plants, despite a significant reduction in height. However, there was a significant decrease in the biomass of main tillers of brushed plants. Crook and Ennos (1996) observed no difference in stem weight between free-standing and supported wheat plants. Goodman and Ennos (1997) also found no difference in total plan dry weight between supported and freestanding maize plants. In dicots, Anten et al. (2010) found a significant reduction in the mass of plantain following either brushing wind treatment. Garner and Bjorkman (1996), also found a decrease in shoot dry mass after brushing treatment.

6.4.3 Mechanical properties

At the end of flowering, only wind treated plants were significantly affected by treatment. Wind treated plants had a significantly reduced stem diameter, both internally and externally, which resulted in reductions in bending rigidity - this was significantly less than the static plants, but not with brushed plants. When treated plants were more often filled with a pith then either static or brushed, though this appears not to have affected the overall stem cross-sectional area. Treatment had no significant effect on the Young's modulus of plants grown outside when measured at the end of flowering. In comparison, the experiments in both Chapter 4 and 5, found a significant reduction in cross section area and bending strength.

When the stem mechanical properties of senesced mature plants were measured both the wind and brushed plants had significantly reduced outer diameter, but only brushed plants have a significantly reduced cross-sectional area. Reductions in both the outer diameter and cross section area were found to be reduced following treatment in both Chapter 4 and 5. There was no effect on either bending strength or bending rigidity, but plants from both treatments had an increased in Young's modulus of the third internode (as was also seen in Chapter 5).

It appears that brushing did not add to the effects of wind in young plants (measured at the end of flowering) and moreover had less of an effect than wind alone. Crock and Ennos (1996) found a significant increase only in bending rigidity at the same position on the stem of free-standing plants. Reductions in the bending rigidity seen in the current experiment is due to reductions in outer stem diameter, rather than changes in Young's modulus. Goodman and Ennos (1997) also found no significant decrease in either bending rigidity or Young's modulus of free-standing plants. Smith and Ennos (2003), found that wind and flexing had opposite effects on the bending rigidity of sunflower stems, with an increase following flexing and decrease following wind exposure. However, flexing in addition to wind had no significant effect compared with control plants. Increased airflow can cancel out the effects of stem flexing on mechanical properties, as each has an opposite effect on the plant. Smith and Ennos (2003), suggested that there are trade-offs between mechanical and hydraulic functions of the stem, as strength decreases when conductivity increased and vice versa. Grace and Russell (1977) observed an increase in the Young's modulus of fescue plants exposed wind, while Goodman and Ennos (1996) found an increase in Young's modulus after flexing maze

plants. It seems that the response of plants to mechanical stimulation, at least in regard to the mechanical properties, is complex and requires allot more teasing-apart, as the situation is further complicated by the addition of airflow around the plant.

6.4.4 Grain yield

Main tiller spikes from all remaining plants were analysed by µCT scanning and image analysis as detailed in the methodology section. Neither treatments had a significant effect on the number of grains per main tiller spike, whereas individual grain volume was significantly reduced. Brushed plants also had significantly smaller grains than wind treatment. However, there was no difference in the total volume per main tiller spike either between treatments and static plants or between treatments. The previous two experiments saw a reduction across all three measurements when plants were grown in a greenhouse. Yield differences between plants grown in a greenhouse and those grown outside suggests that reductions in individual grain volume are likely to de due to limitations in available assimilates during grain filling as a result of mechanical treatment. Differences in grain number may therefore be due to fertility of florets, but not as a result of brushing treatment.

Analysis of spikes and grains using µCT scanning is a relatively new tool, so there are few studies to compare changes in grain size, shape and number with. Previous studies have focused on the effects of drought and heat stress on grain number and size. Transient increases in temperature have a dramatic effect on wheat yield, especially if it occurs during particularly sensitive stages of the plants developments, such as during early stages of meiosis and during anthesis (Hughes et al. 2017, Draeger and Moore 2017).

Drought also affects yield and may have severe implications if it occurs during reproductive phases. In all experiments conducted here, high moisture levels were maintained by regularly watering the plants, so it is unlikely that they may have suffered from drought stress. As temperatures were not closely measured during experiments, the effect of high temperatures on flowering or fertility cannot be ruled out. However, this would affect all plants equally, both static and treated and therefore the effects on grain seen here are clearly due to mechanical treatment.

Prior to anthesis, stem reserve accumulation is a significant factor affecting flower and grain development under stress conditions (Blum 1998). Reductions in carbon assimilation during stem elongation due to drought stress lead to a reduction in the storage capacity of the stems (Barnabás, Jäger, and Fehér 2008). This suggests that reduction in grain size seen in mechanically treated plants may be linked to changes associated with the stems, such as a reduction in length and diameter.

Though not significant, the increase in grain count of treated plants off-set the decrease in individual grain volume to result in no overall difference in total grain volume. The increase in grain count seen in this experiment could be related to the death of many of the tillers prior to anthesis (Foulkes et al. 2010). Tillers compete for the plants resources and termination of infertile or small tillers means that more energy can be focused on the production of grain in the fertile spikes (Yang et al. 2019).

6.4.5 Conclusions

Brushing wind exposed plants did result in a further decrease in individual grain volume, but this did not affect total grain volume for the spike. The results presented here are novel and the effect of mechanical stress on individual wheat grain volume and main tiller grain count has not been reported before.

Plants grown outside responded to mechanical treatment in much the same way as those grown in the greenhouse, though differences in trait measurements between plants grown in the greenhouse and out may be related to the more variable environmental conditions of the natural environment.

There appears to be very little difference between plants grown under natural wind conditions and those that received supplementary brushing. Airflow may have an opposite effect to mechanical treatment but bending induced by the wind is enough to saturate the plants response and is not further enhanced by additional brushing.

Chapter 7

7 General discussion and conclusions

7.1 Effect of brushing on height

The most frequently reported response to mechanical treatment is a reduction in vertical growth, resulting in shorter plants than those that were not treated. Across all the experiments described in this study, there was a clear and significant reduction in height in response to brushing, simulated wind and outdoor wind treatments. One brushstroke was enough to cause a significant difference in height during the period of treatment, though it was not enough to cause a long-lasting effect. Treatment using 3, 12 and 15 brushstrokes resulted in lasting reductions in height in the second experiment, while 20 brushstrokes also had a lasting effect in the third and fourth experiments. This suggests that wheat needs a significant amount mechanical stimulation to bring about a lasting effect on height. A study on the effect of increasing doses of rubbing treatment on rice found a significant reduction in height compared with controls from weeks 3 to 5, but no difference after 5 weeks (Zhao et al. 2018). The reduction in height seen in chapter four followed a roughly curvilinear pattern, where the response increased with increasing dose. The same pattern of response was seen

by Telewski and Pruyn (1998) in flexed *Ulmus Americana* seedlings with increasing doses of flexing.

In chapter five, the effect of brushing plants of different ages was studied. When treatment started at either 2 or 4 weeks post emergence, treated plants were significantly shorter than untreated when height was measured at the end of treatment. Starting treatment at 6 weeks also reduced height. However, at the end of flowering, plants that started treatment at 4 weeks had the greatest height difference with controls, whereas differences for 2 and 6 weeks plants was less pronounced. For all the treatments studied there was a noticeable difference in the response of internodes. When treatment began at 2 weeks after emergence, the length of internodes 1, 2, and 3, was most significantly reduced, and for 4 weeks, it was internodes 2, 3, and 4. For 6 week plants, the lengths of internodes 3 and 4 were only slightly reduced. A lack of studies comparing the response of plants of different ages to mechanical treatment makes drawing conclusions tricky, but studies on recently emerged seedlings (Steucek and Gordon 1975) and stresses such as drought and high temperature indicate that young tissues are most sensitive to stress. It does indeed look like young tissues in the stem are most affected by the mechanical treatment, as demonstrated in the patterns of response of the internodes. Internodes most affected by treatment are those that were undergoing expansion during the period of mechanical treatment. This is because elongation is halted in response to a mechanical stimulus (Coutand 2010). Jaffe et al. (1985) found that mechanical perturbation of maize seedlings resulted in a cessation of elongation for 38 minutes. After a pause, elongation is progressively restored (Coutand et al. 2000). However, once growth resumes, there is no compensation to make up for the halt in elongation, therefore resulting in shorter plants than those that did not receive the stimulation.

Plants grown outside showed significant reductions in height following wind exposure and additional brushing, both after treatment and at the end of flowering. Crook and Ennos (1996) found no difference in height between staked and free-standing wheat plants, but this may be due to pre-exposure of the plants to natural (wind) conditions before the experiment began.

In the final experiment, where plants were exposed to natural and ambient wind conditions, both those that received additional brushing and those that did not were significantly shorter than those that were sheltered and received no treatment. However, there was no significant effect of brushing in addition to exposure to natural wind conditions. This suggests that the levels of brushing used did not add to the effect of the wind and that the removal of mechanical stimulation by sheltering plants results in taller plants. Without mechanical treatment form the wind, these sheltered plants may be more at risk of stem failure if exposed to high strength winds due to an increased centre of gravity. In the horticultural industry where vegetable seedlings are grown in greenhouses with near-static air, mechanical conditioning is used to control plant height (Baden and Latimer 1992, Björkman 1998). The results of the experiment in chapter 6 suggest that for wheat, the brushing methodology used is not sufficient for controlling crop height any more than conditioning provided by wind induced bending, when plants are grown outdoors.

7.2 Stem diameter

In this study, stem diameter was up to 23% narrower in plants that received brushing treatment and 14% narrower where plants grown outdoors were flexed by the wind. Previous

studies have reported an increase in stem diameter following mechanical treatment. Goodman and Ennos (1997) reported that the stems of free standing sunflower plants were 10-11% wider than supported plants. Conversely a decrease in the diameter of cauliflower and tomato stems has been observed (Biddington 1986). Studies on monocots have also reported an increase in stem diameter including in rice (Zhao et al. 2012), approximately 15% in sorghum (Lemloh et al. 2014) and 11% in maize (Goodman and Ennos 1997). However, one study by Goodman and Ennos (1996) found an 8% reduction in the stem diameter of maize following flexing.

All of the experiments conducted in this thesis found a significant decrease in the diameter of wheat stems following mechanical treatment. This was also frequently coupled with reductions in bending strength and bending rigidity, and an increase in Young's modulus. Goodman and Ennos (1996) did not observe any change in mechanical properties associated with a reduction in stem diameter.

Cleugh et al. (1998) remarked that reductions in the size of plant parts results in smaller forces generated by their motions. Furthermore, a study by Gillies et al. (2002) found that exposing fountain grass to increasing windspeeds resulted in a decrease in both frontal area and porosity. Fountain grass appears to re-configure to a more aerodynamic form and whole-plant drag co-efficient is reduced following wind exposure. Reductions in vertical growth and stem diameter produces a plant with reduced surface-area, and consequently there is less area for wind to act against, thus reducing the chance of bending and breaking. This streamlining type of effect could be an adaptive strategy to reduce drag effect and survive strong winds. Further work is needed to clarify if this is a streamlining process and if this

affects how the plant behaves under windy conditions such as how forces in the stem are dampened.

7.3 Tillers and flowers

Due to the lack of studies on the effect of mechanical treatment on monocots, compared with dicots, there is very little information on how mechanical treatment affects Tillering. Tillering was found to increase under all treatments, and the effect was greater with increasing doses of brushing. However, tillers in older plants were not significantly affected by treatment, as seen in chapter five. Plants that were brushed produced up to 61% more tillers than those that were untreated, while plants exposed to natural wind conditions without extra brushing had up to 37% more.

Treatment had no significant effect on tillering in perennial ryegrass (Wang et al. 2010), however, Zhao et al. (2018) found a 21-23% increase in tillering in rice following rubbing treatment. Stresses such as drought and high temperature reduce tiller numbers under intense stress (Goudarzi and Pakniyat 2008), but an increase in tillering was observed under mild stress.

Management practices such as seeding density, growth regulators and rolling also affect tillering (Peltonen and Peltonen-Sainio 1997). Rolling is a management practice applied to crops at early stages of growth, with the primary objective of compacting the soil to reduce root lodging. This process also breaks the uniculm growth habit of cereals and results in increased tillering (Peltonen-Sainio and Peltonen 1997). Berry et al (2002) found that applying rolling treatment to plants undergoing tillering reduced lodging, but applying treatment once

stem extension had begun damages the growing stem and had no effect on lodging (Berry 2019). It is likely that the practice of Mugifumi (Iida 2014) has similar effects to rolling, and is known to increase tillering. Berry suggests that the increase in tillering is a consequence of breaking the stem, which is not the cause of an increase in tillering when plants are brushed. However, increased tiller numbers per plant is not necessarily a positive, even if it results in more flowering spikes. Fewer tillers is associated with lodging resistance (Khobra et al. 2019). Greater numbers of tillers increases the chance of the plant lodging due to an increase in the bending moment (Tripathi et al. 2003, Berry et al. 2004).

The reason for the increase in tillering seen in this study may be due to a disturbance of the leaf which lies over the top of the tiller bud. Liu and Finlayson (2019) found that removing the overlying leaf and sheath stimulated tiller bud growth in sorghum. They suggest that tiller bud growth is suppressed by mechanical signals generated by the overlying leaf. Once the leaf is removed, the mechanical forces experienced by the tiller bud are removed, therefore signalling for it to grow more rapidly. Earlier studies using wheat also suggested that physical pressure around the base of the plant plays a role in the regulation of tiller development (Williams and Metcalf 1975). Additionally, removal of the overlying leaf changes the light environment of the tiller bud, which may also play a role in promoting tiller growth (Liu and Finlayson 2019).

In both the dose-response and age-response experiments, treated plants that saw an increase in tillering also had an increase in the number of flowering spikes. However, in the final experiment, both wind treated and plants that received additional brushing did not see an increase in flowers. Czepak et al. (2019) reported that though tiller numbers were slightly

elevated, treatment did not have a significant effect in tillering in field grown wheat following mechanical treatment, and there was no difference in flower numbers. The lack of difference in the final experiment (chapter 6) could be due to fluctuating or extreme environmental conditions that promoted tiller death, or factors influenced by an increase in airflow.

7.4 Grain

The effect of mechanical treatment on wheat grain size and number has not been studied previously. Main tiller individual grain volume was reduced across all experiments and treatments, except when treatment was applied to plants at 6 weeks post emergence. Treating these older plants resulted in a minor, but significant increase in individual grain volume. Effects on main tiller individual grain numbers were significant for plants grown in the greenhouse, but not when grown outside (both wind and additional brushing treatments). When plants were grown under controlled conditions, treated plants saw a significant reduction in grain numbers in main tiller spikes. However, this effect was not significant when treatment began at 4 or 6 weeks post emergence. The overall effects on individual grain volume and grain count resulted in significant reductions in main tiller total grain volume for plants grown in the greenhouse. When plants were grown outside there was no significant effect on total grain volume. Grain number and size were affected by the mechanical treatment during flower formation, meiosis and grain filling.

Formation of the floret occurs just before or coinciding with initiation of stem extension. At this stage, the floret is highly sensitive to stress. Temperatures of more than 30°c can lead to complete sterility of the flower (Owen 1971). Meiosis to form the pollen and embryo sac occurs during the booting stage, and this is the most stress sensitive stage of reproductive

development. Stress during floret formation and meiosis significantly affects grain numbers and limits potential yield. Grain number reduces significantly following water stress applied during spike growth and emergence (Acevedo, Silva, and Silva 2002). Drought stress applied during tillering also resulted in a reduction in grain numbers (Blum et al. 1990).

In most of the experiments conducted in this present study, grain count was significantly reduced. This suggests that mechanical stress is affecting the plants during flower or grain formation. When treatment began at 2 weeks after emergence in the greenhouse experiments, treatment was applied during tillering and stem extension. Whereas, when treatment began at 4 weeks, there was no significant effect on grain count. Treatment of these plants overlapped the end of tillering, and covered stem extension, and booting. This suggests that mechanical stress may be affecting flower formation more than meiosis, resulting in reduced numbers of grains in main tiller spikes. However, there was no difference in the grain count of plants grown outside. This could be due to increased environmental stresses (other than mechanical) such as high temperature. These stresses would have effected all plants, including controls, and the effect may have been greater than that of the mechanical stimuli. More research is needed to find out how and why there was a reduction in grain numbers following mechanical treatment.

Individual grain volume was significantly reduced by brushing across all experiments, though starting treatment at 6 weeks post emergence had no significant effect. Grain volume was reduced for plants grown in the greenhouse and outside. Grain volume is most significantly influenced by assimilate supply, but stresses applied during grain filling also reduce volume. Water stress in particular reduces volume, by causing grains to shrivel, and this can be

exacerbated by increased temperatures which usually accompany drought stress (Gooding 2010).

In wheat, stem reserves provide an important source of carbon for grain filling (Acevedo, Silva, and Silva 2002). Stems store carbohydrates in the form of starch, glucose, fructose and sucrose, but the greatest reserves are fructans. During the stem extension stage, stress may affect carbon assimilation, thus reducing storage in the stems (Blum 1998). Furthermore, the ability of the stem to act as a store is determined by stem length and weight density. Stem reserve mobilisation during grain filling is also affected by environmental stresses. Under favourable conditions, stems contribute to 20% of grain yield (Blum 1998). Mechanical treatment reduced both stem length and diameter. Individual grain volume was reduced across all of the experiments conducted, suggesting that mechanical stress may be affecting stem assimilate reserves. This is therefore leading to a reduction in grain filling. To find out if this is the case, total non-structural carbohydrate (TNC) in the stems of mechanically treated and untreated plants could be studied. Reductions in both TNC and grain volume would therefore indicate that reductions in grain volume are indeed due to reductions in stem storage and assimilates.

Stem storage also provides significant N reserves for grain filling (Tahir and Nakata 2005), therefore it would also be interesting to look at grain quality in response to mechanical treatment.

7.5 Mechanical properties of the stem

When brushing treatment was initiated at 2 weeks post emergence, plants had significantly narrower stems that plants that did not receive treatment at the end of flowering. Treatment also reduced stem cross-section area in both the dose-response and age-response experiments, but not significantly in plants grown outside. Both of the greenhouse-based experiments also saw a reduction in bending strength and bending rigidity with no effect on the Young's modulus at the end of flowering. When senesced stems were analysed, only plants from the dose-response experiment saw a decrease in bending strength and bending rigidity. Results for the Young's modulus of senesced plants was variable when plants were treated with 20 brushstrokes from 2 weeks post emergence. A significant increase in Young's modulus was seen in both chapter 5 and 6, but there was no difference seen in chapter 4. The plants studied in chapter 6 did not follow the same pattern of response for bending strength and bending rigidity as plants grown in the greenhouse. However, all plants had a significant increase in the modulus of elasticity.

The Young's modulus (E) measured in this study is estimated from measurements produced by 3-point bending tests, and therefore is an aggregate of all of the stem materiel. E here is calculated based on the second moment of area (I), which is estimated with an assumption of a circular cross-section and therefore is a property of the geometry (shape) of the stem. I is calculated to the fourth power of the radius, therefore any changes in radius has a huge effect on I and therefore E also. It is important to note that by the methods used in this present study, E and I are estimates and subject to error. However, bending rigidity (EI) is measured directly using the initial slope of the force/deflection curve produced during the 3-

point bending test. The result is that EI is a more reliable measurement of the stems mechanical properties.

Crook and Ennos (1996) reported that the bending strength of wheat plants was greater if left unsupported whereas supported plants were about 20% weaker. Lemloh et al. (2014) noted a 49.7% and 71.7% increase in bending strength following flexing treatment in sorghum. Maize plants that were able to move and sway in the wind had a 29% greater bending rigidity than those that had been supported (Goodman and Ennos 1997). However, those studies also reported an accompanying increase in stem diameter. Goodman and Ennos (1996) reported that flexed plants were not significantly different in strength to supported maize plants, though there was a reduction in stem diameter in maze following mechanical treatment, which is closer to the results seen in previous chapters. Smith and Ennos (2003), found that wind and flexing had opposite effects on the bending strength of sunflower. They noted an increase following flexing, and a decrease following wind exposure, but no difference compared with controls when both treatments were applied. The effect of airflow can cancel out the effects of bending caused by brushing.

Reductions in stem diameter results in a decrease in the second moment of area of the stem, therefore reducing stem stiffness (Kokubo, Kuraishi, and Sakurai 1989, Coutand 2010). It is common to see a decrease in strength accompanying an increase in diameter, which indicates that stem thickness is not the main determining factor of mechanical properties. Whereas, reductions in bending rigidity are directly related to reductions in stem diameter and cross-section area (Wang et al. 2006). Some studies have found an increase in stem stiffness, with an accompanying decrease in Young's modulus following mechanical treatment (Coutand

2010). Crook and Ennos (1996) observed an increase in the Young's modulus of free-standing wheats stems at 10cm from the base, though there was no difference further up the stems, whereas Goodman and Ennos (1996) found no difference in Young's modulus. The Young's modulus of plant stems is an important factor in the lodging of wheat plants, as an increase in the Young's modulus means that more force is needed to bend the stem. The Young's modulus does not appear to be quite as variable under increased airflow and mechanical treatment. Grace and Russell (1977), noted an increase in the Young's modulus of wind exposed fescue, while Goodman and Ennos (1996), found an increase in the Young's modulus of wheat plants following flexing treatment. However, the effect of increased airflow does seem to complicate the situation, and more research is needed to separate the effects on mechanical properties in wheat.

Despite decreases in stem diameter and cross-section area, there are changes in mechanical properties, including an increase in Young's modulus. Mechanical properties of the stem are not influenced by internode length and diameter alone. An analysis of stem tissues and cell wall composition following treatment could help understand more about the strength of the stem and how it reacts to mechanical stress.

Furthermore, improvements in the measurement of mechanical properties of plant materiel would allow more accurate measurements (Nelson et al. 2019). The 3-point bending machine used in these experiments had a limited resolution of strain measurement, resulting in some measurements with very high standard deviations. Repeating these experiments using improved measurement techniques could help refine and improve the ideas presented here.

Mechanical properties of the stem are an important factor influencing the plants susceptibility to stem and root lodging. Lodging was described as the permanent displacement of stems from their upright position by Pinthus (1974) and results in crop losses of up to 80% (Berry et al. 2004). Lodging occurs when the force of the wind on the plants surface overcomes the strength of the stem or its anchorage, resulting in a bucking or breakage of the stem or a failure of root anchorage. A study by Berry et al. (2000) noted that the most robust wheat varieties in the study required gusts of 26-28 m/s to cause lodging. While the primary factor causing lodging is wind speed, rain, soil-type, topography, disease, and crop husbandry practices also influence the chance of a crop lodging. Root lodging is the predominant form and is influenced by stem and root characteristics but also soil factors. Stem lodging is less common, but strongly influenced by stem morphology, biochemistry, and biomechanics.

Stem failure is dependent on materiel strength, external stem radius and stem wall thickness (Berry et al. 2004). The stiff stems of modern varieties of wheat are able to resist buckling, and therefore the occurrence of stem lodging, however, oscillations created by the force of wind on the stem are transferred to the plants base and roots, increasing the likelihood of root lodging (Crook and Ennos 1994). Strong and stiff stems are able to resist buckling.

Berry et al. (2004) suggests that it is unlikely that wheat plants can be bred or managed to be completely lodging proof using traditional agronomic strategies and methods of breeding. Opportunities for increasing stem strength and reducing plant height appear to have been maximised, and agronomic strategies have also reached an optimum for stem trait management. However, there may be room for further development of root systems in order

to reduce root lodging through increasing anchorage strength by breeding and careful crop management.

Root biomass was measured in preliminary experiments, but not considered further in the later experiments, which was an oversight. In preliminary experiments, root biomass was significantly greater in mechanically treated plants, though the root system was not further investigated. Crook and Ennos (1996), Goodman and Ennos (1996, 1997) and Wang et al. (2010) noted changes in the root system in response to mechanical treatment that may be beneficial in resisting lodging. It would therefore be interesting to repeat the dose-response, age-response and outdoor experiments with a focus on observing the root system, including size, shape and distribution of roots, but also mechanical and anchorage properties.

7.6 Mechanosensing – how plants sense and respond to the mechanical stimuli

Mechanosensing is the process by which plants sense and respond to a mechanical stimuli. It includes both sensing of mechanical signals coming from within the plant, created by the expansion or contraction of neighbouring cells, but also mechanical signals originating from external sources

Two methods of mechanoperception have been suggested: Firstly, stretch-activated channels might be activated by mechanical stimulation of the call membrane, which leads to an ion flux change. Secondly, proteins that link the extra-cellular matrix, plasma membrane and/or cytoskeleton may act as mechanoreceptors (Braam 2005). Though it may in-fact be a mixture of both methods. It is understood that the plant is sensing the strain of the mechanical load rather than the stress (Braam 2005). The process of mechanosensing can be generally

summarised as; 1. The plant bares the load, which is 2. Sensed by mechanosensitive tissues, followed by 3. A transduction of the signal from the mechanosensitive tissues and results in 4. Responses by the plant, such as a halt in growth (Onoda and Anten 2011).

For a long time, it has been recognised that cystolic Ca2+ is an important secondary messenger and increases rapidly after mechanical stimulation. Reactive oxygen species (ROS) also increase rapidly following mechanical stimulation and there is evidence that they may regulate Ca2+ channels, suggesting that the two are closely linked (Braam 2005). Auxin and Ethylene may also have roles in the response to mechanical stimulation (Jaffe and Biro 1979, Boyer and Chapelle 1979, Hofinger et al. 1980, Jaffe 1980).

Five touch-inducible (TCH) genes have been discovered in Arabidopsis which are strongly and rapidly up-regulated in expression following mechanical stimulation such as touching leaves, rubbing, wind, and water spray (Braam et al. 1997, Braam 2005). Three of these genes are; TCH1, which encodes Calmodulin, a major Ca2+ receptor in cells, TCH3, which may be involved in cell or tissue reinforcement or cell expansion play a role in modifying the plant cell wall, and TCH4 is a xyloglucan endotransglucosylase (XET), which may be involved in strengthening the cell wall of non-growing cells (Braam et al. 1997).

Most of what is known on Mechanosensing has come from studies on dicots, and most notably Arabidopsis. A touch-regulated Lipoxygenase gene has been identified in wheat (Mauch et al. 1997), but TCH genes in wheat have yet to be identified. Much more research is needed to better understand the regulation of mechanosensing in monocots and to identify if similar molecular mechanisms are involved in mechanical stimulation responses in wheat.

This study demonstrated that wheat growth was significantly affected by brushing treatment, so it would be interesting to look more closely at the mechanisms and genes involved in the response.

7.7 Conclusion

To conclude, wheat responds strongly to mechanical treatment applied by brushing above-ground plant parts. Brushing resulted in significant reductions in vertical growth which lasted to maturity. Brushing also resulted in a reduction in stem diameter, and therefore reduced associated stem mechanical properties. Changes in these measurements may also be linked to modifications in tissues and call walls, which requires further study. Treatment reduced main tiller grain count, and this may be a result of the application of mechanical stress during floret formation or meiosis. Reductions in grain volume across all treatments may be linked to reductions in stem carbon storage and related to reductions in stem diameter and length caused by brushing treatment.

8 References

Abhinandan, Kumar, Logan Skori, Matija Stanic, Neil MN Hickerson, Muhammad Jamshed, and Marcus A Samuel. 2018. "Abiotic Stress Signaling in Wheat–An Inclusive Overview of Hormonal Interactions During Abiotic Stress Responses in Wheat." *Frontiers in plant science* 9.

Acevedo, E, P Silva, and H Silva. 2002. "Wheat growth and physiology." *Bread Wheat, Improvement and Production* 30.

AHDB. 2016. AHDB Recommended Lists for cereals and oilseeds 2016/17. https://projectblue.blob.core.windows.net/media/Default/Imported%20Publication%20Docs/AHDB%20Cereals%20&%20Oilseeds/Varieties/RL2016-17/Recommended%20Lists%20for%20cereals%20and%20oilseeds%202016-17.pdf: HDB Cereals and Oilseeds.

AHDB. 2018. Wheat Growth Guide. In *https://cereals.ahdb.org.uk/media/185687/g66-wheat-growth-guide.pdf*, edited by AHDB Cereals and Oilseeds. Kenilworth, UK.

Anten, Niels, Rafael Alcalá-Herrera, Feike Schieving, and Yusuke Onoda. 2010. "Wind and Mechanical Stimuli Differently Affect Leaf Traits in *Plantago major*." *New Phytologist* 188:554-564.

Anten, Niels, Raquel Casado-Garcia, and Hisae Nagashima. 2005. "Effects of Mechanical Stress and Plant Density on Mechanical Characteristics, Growth, and Lifetime Reproduction of Tobacco Plants." *The American Naturalist* 166 (6):650-660.

Autio, Janne, Irma Voipio, and Taina Koivunen. 1994. "Responses of aster, dusty miller, and petunia seedlings to daily exposure to mechanical stress." *HortScience* 29 (12):1449-1452.

Baden, Sherrod, and Joyce Latimer. 1992. "An Effective System for Brushing Vegetable Transplants for Height Control." *Horticulture Technology* 2 (3):412-414.

Barnabás, Beáta, Katalin Jäger, and Attila Fehér. 2008. "The effect of drought and heat stress on reproductive processes in cereals." *Plant, cell & environment* 31 (1):11-38.

Batey, Ian. 2017. "The diversity of uses for cereal grains." In *Cereal Grains*, 41-53. Elsevier.

Bell, M, and R Fischer. 1994. Guide to Plant and Crop Sampling: Measurements and Observations for Agronomic and Physiological Research in Small Grain Cereals. In *Wheat Special Report No. 32*. Mexico.

Berry, P, M Sterling, J Spink, C Baker, R Sylvester-Bradley, S Mooney, A Tams, and A Ennos. 2004. "Understanding and reducing lodging in cerials." *Advancesin Agronomy* 84.

Berry, PM. 2019. "Lodging resistance in cereals." *Crop Science*:209-227.

Berry, PM, JM Griffin, R Sylvester-Bradley, RK Scott, JH Spink, CJ Baker, and RW Clare. 2000. "Controlling plant form through husbandry to minimise lodging in wheat." *Field Crops Research* 67 (1):59-81.

Berry, PM, and J Spink. 2012. "Predicting yield losses caused by lodging in wheat." *Field Crops Research* 137:19-26.

Berry, PM, JH Spink, JM Griffin, R Sylvester-Bradley, CJ Baker, RW Clare, and RK Scott. 1998. "Research to understand, predict and control factors affecting lodging in wheat." *HGCA Project Report (United Kingdom)*.

Berry, PM, JH Spink, R Sylvester-Bradley, A Pickett, M Sterling, C Baker, and N Cameron. 2002. "Lodging control through variety choice and management." Proceedings of the 8th Home-Grown Cereals Association R & D Conference on Cereals and Oilseeds, pages.

Berry, PM, R Sylvester-Bradley, and S Berry. 2007. "Ideotype design for lodging-resistant wheat." *Euphytica* 154 (1-2):165-179.

Biddington, N, and A Dearman. 1985. "The Effect of Mechanically Induced Stress on the Growth of Cauliflower, Lettuce and Celery Seedlings." *Annals of Botany* 55 (109-119).

Biddington, Norman. 1986. "The Effects of Mechanically-Induced Stress in Plants - A Review." *Plant Growth Regulation* 4:103-123.

Björkman, Thomas. 1998. "Mechanical Conditioning for Controlling Excessive Elongation in Transplants." *Journal of the Japanese Society for Horticultural Science* 67 (6):1121-1123.

Blum, A. 1998. "Improving wheat grain filling under stress by stem reserve mobilisation." *Euphytica* 100 (1-3):77-83.

Blum, A, S Ramaiah, ET Kanemasu, and GM Paulsen. 1990. "Wheat recovery from drought stress at the tillering stage of development." *Field Crops Research* 24 (1-2):67-85.

Boyer, N, and B Chapelle. 1979. "Lithium Inhibition of the Thigmomorphogenetic Response in *Bryonia dioica*." *Plant Physiology* 63 (6):1215-1216.

Braam, Janet. 2005. "In Touch: Plant Responses ro Mechanical Stimuli." *New Phytologist* 165 (2):373-389.

Braam, Janet, and Ronald W Davis. 1990. "Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis." *Cell* 60 (3):357-364.

Braam, Janet, Melissa L Sistrunk, Diana H Polisensky, Wei Xu, Mary M Purugganan, Danuta M Antosiewicz, Paul Campbell, and Keith A Johnson. 1997. "Plant responses to environmental stress: regulation and functions of the ArabidopsisTCH genes." *Planta* 203:S35-S41.

Börnke, Frederik, and Thorsten Rocksch. 2018. "Thigmomorphogenesis–Control of plant growth by mechanical stimulation." *Scientia Horticulturae* 234:344-353.

Campbell, C. A., H. R. Davidson, and G. E. Winkleman. 1981. "EFFECT OF NITROGEN, TEMPERATURE, GROWTH STAGE AND DURATION OF MOISTURE STRESS ON YIELD COMPONENTS AND PROTEIN CONTENT OF MANITOU SPRING WHEAT." *Canadian Journal of Plant Science* 61 (3):549-563. doi: 10.4141/cjps81-078.

Chanda, S, and Y Singh. 2002. "Estimation of Leaf Area in Wheat Using Linear Measurements." *Plant Breeding and Seed Science* 46 (2):75-79.

Chehab, Wassim, Elizabeth Eich, and Janet Braam. 2009. "Thigmomorphogenesis: A Complex Plant Response to Mechano-Stimulation." *Journal of Experimental Botany* 60 (1):43-56.

Chinnusamy, Viswanathan, and Jian-Kang Zhu. 2009. "Epigenetic regulation of stress responses in plants." *Current Opinion in Plant Biology* 12 (2):133-139. doi: https://doi.org/10.1016/j.pbi.2008.12.006.

Cleugh, H, J Miller, and M Böhm. 1998. "Direct Mechanical Effects of Wind on Crops." *Agroforestry Systems* 41:85-112.

Coutand, C, J Julien, B Moulia, J Mauget, and D Guitard. 2000. "Biomechanical study of the effect of a controlled bending on tomato stem elongation: global mechanical analysis." *Journal of Experimental Botany* 51:1813-1824.

Coutand, Catherine. 2010. "Mechanosensing and Thigmomorphogenesis, a Physiological and Biomechanical Point of View." *Plant Science* 179:168-182.

Crook, M, and A Ennos. 1996. "Mechanical Differences Between Free-standing and Supported Wheat Plants *Triricum aestivum* L." *Annals of Botany* 77:197-202.

Crook, MJ, and AR Ennos. 1994. "Stem and root characteristics associated with lodging resistance in four winter wheat cultivars." *The Journal of Agricultural Science* 123 (2):167-174.

Czepak, Marcio Paulo, Valmor Sornberger, Weslley do Rosário Santana, Luã Víthor Chíxaro Almeida Falcão Rosa, Vinicius de Souza Oliveira, Karina Tiemi Hassuda dos Santos, Omar Schmildt, and Edilson Romais Schmildt. 2019. "Mechanical Damage in the Tillering, Development and Productivity of Wheat."

da Costa, Ricardo MF, Sivakumar Pattathil, Utku Avci, Ana Winters, Michael G Hahn, and Maurice Bosch. 2019. "Desirable plant cell wall traits for higher-quality miscanthus lignocellulosic biomass." *Biotechnology for biofuels* 12 (1):85.

Darwin, Charles, and Francis Darwin. 1880. *The power of movement in plants*: Appleton.

de Langre, Emmanuel. 2008. "Effects of Wind on Plants." *Annual Review of Fluid Mechanics* 40:141-168.

deLangre, Emmanuel. 2008. "Effects of Wind on Plants." *Annual Review of Fluid Mechanics* 40:141-168.

Değer, Gözde, Mehmet Pakdemirli, Feyza Candan, Selda Akgün, and Hakan Boyacı. 2010. "Strength of wheat and barley stems and design of new beam/columns." *Mathematical and Computational Applications* 15 (1):1-13.

Draeger, Tracie, and Graham Moore. 2017. "Short periods of high temperature during meiosis prevent normal meiotic progression and reduce grain number in hexaploid wheat (Triticum aestivum L.)." *Theoretical and applied genetics* 130 (9):1785-1800.

Easson, D, E White, and S Pickles. 1992. A Study of Lodging in Cereals. In *Research Project No. 52*, edited by HGCA. London: Home Grown Cereals Authority.

Ennos, AR. 1997. "Wind as an ecological factor." *Trends in Ecology & Evolution* 12 (3):108-111.

FAOSTAT. 2019. Crop statistics database. In *FAO Global Statistical Yearbook, FAO Regional Statistical Yearbooks.* http://www.fao.org/faostat/en/#data/QC: Food and Agriculture Organization of the United Nations (FAO).

Finnigan, John. 2000. "Turbulence in plant canopies." *Annual Review of Fluid Mechanics* 32 (1):519-571.

Fischer, RA, and M Stapper. 1987. "Lodging effects on high-yielding crops of irrigated semidwarf wheat." *Field Crops Research* 17 (3-4):245-258.

Ford, M, R Blackwell, M Parker, and R Austin. 1979. "Associations Between Stem Solidity, Soluble Carbohydrate Accumulation and Other Characters in Wheat." *Annals of Botany* 44:731-738.

Foulkes, M John, Gustavo A Slafer, William J Davies, Pete M Berry, Roger Sylvester-Bradley, Pierre Martre, Daniel F Calderini, Simon Griffiths, and Matthew P Reynolds. 2010. "Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance." *Journal of experimental botany* 62 (2):469-486.

Gardiner, Barry, Peter Berry, and Bruno Moulia. 2016. "Review: Wind Impacts on Plant Growth, Mechanics and Growth." *Plant Science* 245:94-118.

Garner, Lauren, and Thomas Björkman. 1996. "Mechanical Conditioning for Controlling Excessive Elongation in Tomato Transplants: Sensitivity to Dose, Frequency, and Timing of Brushing." *Journal of American Horticultural Science* 121 (5):894-900.

Garner, Lauren, and Thomas Björkman. 1997. "Using Impedance for Mechanical Conditioning of Tomato Transplants to Control Excessive Stem Elongation." *Horticultural Science*.

Garner, Lauren, Allen Langton, and Thomas Björkman. 1997. "Commercial Adaptations of Mechanical Stimulation for the Control of Transplant Growth." *Acta Horticulturae* 435:219-230. doi: 10.17660/ActaHortic.1997.435.21.

Gartner, Barbara. 1994. "Root biomechanics and whole-plant allocation patterns: responses of tomato plants to stem flexure." *Journal of Experimental Botany* 45:1647-1654.

Gibson, L. R., and G. M. Paulsen. 1999. "Yield Components of Wheat Grown under High Temperature Stress during Reproductive Growth Contribution 97-453-J of the Kansas Agric. Exp. Stn., Manhattan." *Crop Science* 39 (6):1841-1846. doi: 10.2135/cropsci1999.3961841x.

Gillies, JA, WG Nickling, and J King. 2002. "Drag coefficient and plant form response to wind speed in three plant species: Burning Bush (Euonymus alatus), Colorado Blue Spruce (Picea pungens glauca.), and Fountain Grass (Pennisetum setaceum)." *Journal of Geophysical Research: Atmospheres* 107 (D24):ACL 10-1-ACL 10-15.

Gomez, Francisco E, Geraldo Carvalho, Fuhao Shi, Anastasia H Muliana, and William L Rooney. 2018. "High throughput phenotyping of morpho-anatomical stem properties using X-ray computed tomography in sorghum." *Plant methods* 14 (1):59.

Gooding, Michael Jonathan. 2010. "The effects of growth environment and agronomy on grain quality." In *Cereal Grains*, 393-412. Elsevier.

Gooding, MJ, JPRE Dimmock, J France, and SA Jones. 2000. "Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield." *Annals of applied Biology* 136 (1):77-84.

Goodman, AM, and AR Ennos. 1996. "A comparative study of the response of the roots and shoots of sunflower and maize to mechanical stimulation." *Journal of Experimental Botany* 47 (10):1499-1507.

Goodman, AM, and AR Ennos. 1997. "The responses of field-grown sunflower and maize to mechanical support." *Annals of Botany* 79 (6):703-711.

Gordon, James Edward. 2009. *Structures: or why things don't fall down*: Da Capo Press.

Goudarzi, Mehdi, and Hassan Pakniyat. 2008. "Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits." *J. Agri. Soc. Sci* 4:1813-223504.

Grace, J. 1974. "The Effect of Wind on Grasses. I. Cuticular and Stomatal Transpiration." *Journal of Experimental Biology* 25 (86):542-551.

Grace, J, and G Russell. 1977. "The Effect of Wind on Grasses. III. Influence of Continuous Drought or Wind on Anatomy and Water Relations in *Festuca arundinacea* Schreb." *Journal of Experimental Botany* 28 (103):268-278.

Grace, J, and G Russell. 1982. "The Effect of Wind and a Reduced Supply of Water on the Growth and Water Relations of *Festuca arundinacea* Schreb." *Annals of Botany* 49:217-225.

Grace, J. 1977. *Plant response to wind*. London: Academic Press.

Graham, J. 1983. "Crop Lodging in British Wheats and Barleys." Ph.D., University of Reading.

Gupta, N. K., Sunita Gupta, and Arvind Kumar. 2001. "Effect of Water Stress on Physiological Attributes and their Relationship with Growth and Yield of Wheat Cultivars at Different Stages." *Journal of Agronomy and Crop Science* 186 (1):55-62. doi: 10.1046/j.1439-037x.2001.00457.x.

Harris, Peter. 1986. "Rights and wrongs of rolling." *Arable Farming* 14 (1):31-32.

Henry, Hugh AL, and Sean C Thomas. 2002. "Interactive effects of lateral shade and wind on stem allometry, biomass allocation, and mechanical stability in Abutilon theophrasti (Malvaceae)." *American Journal of Botany* 89 (10):1609-1615.

Hofinger, M, B Chappell, N Boyer, and T Gaspar. 1980. "GC-MS identification and titration of IAA in mechanically perturbed *Bryonia dioica*." *Plant Physiology* 63 (5).

Hondroyianni, E, DK Papakosta, AA Gagianas, and KA Tsatsarelis. 2000. "Corn stalk traits related to lodging resistance in two soils of differing salinity." *Maydica* 45 (2):125-133.

Huber, Heidrun, Jan deBrouwer, Eric von Wettberg, Heinjo During, and Niels Anten. 2014. "More Cells, Bigger Cells or Simply Reorganization? Alternative Mechanisms Leading to Changed Internode Architecture Under Contrasting Stress Regimes." *New Phytologist* 201:193-204. doi: 10.1111/nph.12474.

Hughes, Nathan, Karen Askew, Callum P Scotson, Kevin Williams, Colin Sauze, Fiona Corke, John H Doonan, and Candida Nibau. 2017. "Non-destructive, high-content analysis of wheat grain traits using X-ray micro computed tomography." *Plant methods* 13 (1):76.

Hughes, Nathan, Hugo R Oliveira, Nick Fradgley, Fiona Corke, James Cockram, John H Doonan, and Candida Nibau. 2019. "µ CT trait analysis reveals morphometric differences between domesticated temperate small grain cereals and their wild relatives." *The Plant Journal*.

Iida, Hidetoshi. 2014. "Mugifumi, a beneficial farm work of adding mechanical stress to wheat and barley seedlings." *Frontiers in Plant Science* 5:1-3. doi: 10.3389/fpls.2014.00453

Impey , Louise. 2012 "JB Diego is top selling winter wheat." *Farmers Weekly*.

Jacobs, MR. 1954. "The effect of wind sway on the form and development of Pinus radiata D. Don." *Australian Journal of Botany* 2 (1):35-51.

Jaffe, M. 1973. "Thigmomorphogenesis: The Response of Plant Growth and Development to Mechanical Stimulation." *Planta* 144:143-157.

Jaffe, M. 1976. "Thigmomorphogenesis: A detailed charachterization of the response of beans (*Phaseolus vulgaris* L.) to mechanical stimulation." *Zeitschrift für Pflanzenphysiologie* 77 (5):437-453.

Jaffe, M. 1980. "Morphogenetic Responses of Plants to Mechanical Stimuli or Stress." *BioScience* 30 (4):239-243.

Jaffe, M, and R Biro. 1979. "Thigmomorphogenesis: the effect of mechanical perturbation on the growth of plants, with special reference to anatomical changes, the role of ethylene, and interactions with other environmental stresses." In *Stress physiology in crop plants*, edited by H Mussell and R Staples, 25-69. New York: John Wiley and Sons.

Jaffe, M, and S Forbes. 1993. "Thigmomorphogenesis: The Effect of Mechanical Perturbation on Plants." *Plant Growth Regulation* 12:313-324.

Jaffe, MJ, R Biro, and K Bridle. 1980. "Thigmomorphogenesis: calibration of the parameters of the sensory function in beans." *Physiologia Plantarum* 49 (4):410-416.

Jaffe, Mordecai J, A Carl Leopold, and Richard C Staples. 2002. "Thigmo responses in plants and fungi." *American Journal of Botany* 89 (3):375-382.

Jaffe, Mordecai, Andrew Wakefield, Frank Telewski, Edward Gulley, and Ronald Biro. 1985. "Computer-assisted image analysis of plant growth, thigmomorphogenesis, and gravitropism." *Plant Physiology* 77:722-730.

Jones Jr, J Benton. 2007. *Tomato plant culture: in the field, greenhouse, and home garden*: CRC press.

Khobra, Rinki, Sindhu Sareen, Braj Kishor Meena, Arvind Kumar, Vinod Tiwari, and GP Singh. 2019. "Exploring the traits for lodging tolerance in wheat genotypes: a review." *Physiology and Molecular Biology of Plants*:1-12.

Kokubo, Akira, Susumu Kuraishi, and Naoki Sakurai. 1989. "Culm strength of barley: correlation among maximum bending stress, cell wall dimensions, and cellulose content." *Plant physiology* 91 (3):876-882.

Kong, Eryan, Dongcheng Liu, Xiaoli Guo, Wenlong Yang, Jiazhu Sun, Xin Li, Kehui Zhan, Dangqun Cui, Jinxing Lin, and Aimin Zhang. 2013. "Anatomical and chemical characteristics associated with lodging resistance in wheat." *The Crop Journal* 1:43-49.

Latimer, Joyce. 1991. "Mechanical Conditioning for Control of Growth and Quality of Vegetable Transplants." *HortScience* 26 (12):1456-1461.

Latimer, Joyce. 1998. "Mechanical Conditioning to Control Height." *Horticultural Technology* 8 (4).

Latimer, Joyce, and Paul Thomas. 1991. "Application of Brushing for Growth Control of Tomato Transplants in a Commercial Setting." *Horticultural Technology* Oct/Dec:109-110.

Le, Thang Duong Quoc, Camille Alvarado, Christine Girousse, David Legland, and Anne-Laure Chateigner-Boutin. 2019. "Use of X-ray micro computed tomography imaging to analyze the morphology of wheat grain through its development." *Plant methods* 15 (1):84.

Lemloh, Marie-Louise, Anna Pohl, Eva Weber, Marco Zeiger, Petra Bauer, Ingrid M Weiss, and Andreas S Schneider. 2014. "Structure-property relationships in mechanically stimulated Sorghum bicolor stalks." *Bioinspired Materials* 1 (1).

Liu, Ruixian, and Scott A Finlayson. 2019. "Sorghum tiller bud growth is repressed by contact with the overlying leaf." *Plant, cell & environment* 42 (7):2120-2132.

Liu, Yun, Feike Schieving, Josef F Stuefer, and Niels PR Anten. 2006. "The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant." *Annals of Botany* 99 (1):121-130.

Martone, Patrick T, Michael Boller, Ingo Burgert, Jacques Dumais, Joan Edwards, Katharine Mach, Nick Rowe, Markus Rueggeberg, Robin Seidel, and Thomas Speck. 2010. "Mechanics without muscle: biomechanical inspiration from the plant world." *Integrative and comparative biology* 50 (5):888-907.

Mauch, Felix, Alenka Kmecl, Ulrich Schaffrath, Sandra Volrath, Jörn Görlach, Eric Ward, John Ryals, and Robert Dudler. 1997. "Mechanosensitive Expression of a Lipoxygenase Gene in Wheat." *Plant Physiology* 114:1561-1566.

Mitchell, Cary. 1996. "Recent Advances in Plant Response to Mechanical Stress: Theory and Application." Recent Advances in Plant Responses to Stress: Bridging the Gap between Science and Technology.

Mitchell, Cary A, and Patricia N Myers. 1995. "Mechanical stress regulation of plant growth and development." *Horticultural Reviews* 17:1-42.

Monshausen, Gabriele, and Simon Gilroy. 2009. "Feeling Green: Mechanosensing in Plants." *Trends in Cell Biology* 19 (5):228-235.

Morel, P, L Crespel, G Galopin, and B Moulia. 2012. "Effect of Mechanical Stimulation on the Growth and Branching of Garden Rose." *Scientia Horticulturae* 135:59-64.

Moulia, Bruno. 2013. "Plant biomechanics and mechanobiology are convergent paths to flourishing interdisciplinary research." *Journal of experimental botany* 64 (15):4617-4633.

Nakajima, Takashi, Megumi Yoshida, and Kenta Tomimura. 2008. "Effect of lodging on the level of mycotoxins in wheat, barley, and rice infected with the Fusarium graminearum species complex." *Journal of General Plant Pathology* 74 (4):289.

Neel, PL, and RW Harris. 1971. "Motion-induced inhibition of elongation and induction of dormancy in Liquidambar." *Science* 173 (3991):58-59.

Nelson, Nathanael, Christopher J Stubbs, Ryan Larson, and Douglas D Cook. 2019. "Measurement accuracy and uncertainty in plant biomechanics." *Journal of Experimental Botany* 70 (14):3649-3658. doi: 10.1093/jxb/erz279.

Niklas, Karl J. 1998. "Effects of vibration on mechanical properties and biomass allocation pattern of Capsella bursa-pastoris (Cruciferae)." *Annals of Botany* 82 (2):147-156.

Office, Met. 2020. "UK climate averages, Trawsgoed 1981-2010." https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcm1y0vvu.

Onoda, Yusuke, and Niels Anten. 2011. "Challenges to Understanding Plant Responses to Wind." *Plant Signaling and Behavior* 6 (7):1057-1059. doi: 10.4161/psb.6.7.15635.

Owen, PC. 1971. "Responses of a semi-dwarf wheat to temperatures representing a tropical dry season. II. Extreme temperatures." *Experimental Agriculture* 7 (1):43-47.

Paul-Victor, Cloé, and Nick Rowe. 2010. "Effect of Mechanical Perturbation on the Biomechanics, Primary Growth and Secondary Tissue Development of Inflorescence Stems of *Arabidopsis thaliana*." *Annals of Botany*. doi: 10.1093/aob/mcq227.

Peacock, K, and A Berg. 1994. "Effect of mechanical stress on sunflower (Helianthus annuus L.) hypocotyl growth." *Annals of Botany* 74:661-666.

Peltonen, Jari, and Pirjo Peltonen-Sainio. 1997. "Breaking uniculm growth habit of spring cereals at high latitudes by crop management. II. Tillering, grain yield and yield components." *Journal of Agronomy and Crop Science* 178 (2):87-95.

Peltonen-Sainio, Pirjo, and Jari Peltonen. 1997. "Breaking uniculm growth habit of spring cereals at high latitudes by crop management. I. Leaf area index and biomass accumulation." *Journal of Agronomy and Crop Science* 178 (2):79-86.

Pigliucci, Massimo. 2002. "Touchy and Bushy: Phenotypic Plasticity and Integration in Response to Wind Stimulation in Arabidopsis Thaliana." *Journal of Plant Science* 163 (3):399-408.

Pinthus, Moshe J. 1974. "Lodging in wheat, barley, and oats: the phenomenon, its causes, and preventive measures." In *Advances in agronomy*, 209-263. Elsevier.

Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, W. K. Cornwell, J. M. Craine, D. E. Gurvich, C. Urcelay, E. J. Veneklaas, P. B. Reich, L. Poorter, I. J. Wright, P Ray, L. Enrico, J. G. Pausas, A. C. deVos, N. Buchmann, G. Funes, F. Quétier, J. G. Hodgson, K. Thompson, H. D. Morgan, H. ter Steege, M. G. A. van der Heijden, L. Sack, B. Blonder, P. Poschlod, M. V. Vaieretti, G. Conti, A. C. Staver, S Aquino, and J. H. C. Cornelissen. 2013. "New Handbook for Standardised Measurement of Plant Functional Traits Worldwide." *Australian Journal of Botany*. doi: http://dx.doi.org/10.1071/BT12225.

Retuerto, Ruben, and I Woodward. 1992. "Effects of Windspeed on the GRowth and Biomass Allocation of White Mustard *Sinapis alba* L." *Oecologia* 92:113-123.

Robertson, Daniel, Simeon Smith, Brian Gardunia, and Douglas Cook. 2014. "Improved method for accurate phenotyping of corn stalk strength." *Crop Science* 54:2038-2044.

Russell, G, and J Grace. 1978a. "The Effect of Wind on Grasses. IV. Some Influences of Drought or Wind on *Lolium perenne*." *Journal of Experimental Botany* 29 (208):245-255.

Russell, G, and J Grace. 1978b. "The Effect of Wind on Grasses. V. Leaf Extension, Diffusive Conductance, and Photosynthesis in the Wind Tunnel." *Journal of Experimental Botany* 29 (112):1249-1258.

Russell, G, and J Grace. 1979. "The Effect of Windspeed on the Growth of Grasses." *Journal of Applied Ecology* 16:507-514.

Schneider, Caroline A, Wayne S Rasband, and Kevin W Eliceiri. 2012. "NIH Image to ImageJ: 25 years of image analysis." *Nature methods* 9 (7):671-675.

Schnelle, Michael, Dean McCraw, and Timothy Schmoll. 1994. "A Brushing Apparatus for Height Control of Bedding Plants." *HortTechnology* 4 (3):275- 267.

Schrader, Wayne L. 2000. *Using transplants in vegetable production*: UCANR Publications.

Schrank, AR. 1944. "Relation between electrical and curvature responses in the Avena coleoptile to mechanical stimuli." *Plant physiology* 19 (2):198.

Schrank, AR. 1950. "Plant tropisms." *Annual Review of Plant Physiology* 1 (1):59-74.

Scott, DI, AR Tams, PM Berry, and SJ Mooney. 2005. "The effects of wheel-induced soil compaction on anchorage strength and resistance to root lodging of winter barley (Hordeum vulgare L.)." *Soil and Tillage Research* 82 (2):147-160.

Shah, Adnan Noor, Mohsin Tanveer, Atique ur Rehman, Shakeel Ahmad Anjum, Javaid Iqbal, and Riaz Ahmad. 2016. "Lodging stress in cereal—effects and management: an overview." *Environmental Science and Pollution Research*:1-16.

Shah, NH, and GM Paulsen. 2003. "Interaction of drought and high temperature on photosynthesis and grain-filling of wheat." *Plant and Soil* 257 (1):219-226.

Shewry, Peter R. 2009. "Wheat." *Journal of experimental botany* 60 (6):1537-1553.

Smith, V, and A Ennos. 2003. "The effects of air flow and stem flexure on the mechanical and hydraulic properties of the stems of sunflowers *Helianthus annuus* L." *Journal of Experimental Botany* 54:845-849.

Sterling, Mark, Chris Baker, and Peter Berry. 2003. "An experimental analysis of the wind induced failure of wheat crops." Wind Effects on Trees, University of Karlsruhe, Germany, September 16-18.

Sterling, Mark, Chris Baker, Peter Berry, and A Wade. 2003. "An experimental investigation of the lodging in wheat " *Agricultural and Forest Meteorology* 119:149-165.

Steucek, GL, and LK Gordon. 1975. "Response of wheat (Triticum aestivum) seedlings to mechanical stress." *Botanical Gazette* 136 (1):17-19.

Strange, Harry, Reyer Zwiggelaar, Craig Sturrock, Sacha J Mooney, and John H Doonan. 2015. "Automatic estimation of wheat grain morphometry from computed tomography data." *Functional Plant Biology* 42 (5):452-459.

Sönnichsen, N. 2020. Average wind speed in the United Kingdom (UK) 2001-2018. In *https://www.statista.com/statistics/322785/average-wind-speed-in-the-united-kingdom-uk/*, edited by Statista.

Tahir, ISA, and N Nakata. 2005. "Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling." *Journal of Agronomy and Crop Science* 191 (2):106-115.

Telewski, Frank W. 2012. "Is windswept tree growth negative thigmotropism?" *Plant science* 184:20-28.

Telewski, Frank W, and Michele L Pruyn. 1998. "Thigmomorphogenesis: a dose response to flexing in Ulmus americana seedlings." *Tree physiology* 18 (1):65-68.

Thompson, J. 1974. "The Effect of Wind on Grasses. II. Mechanical Damage in *Festuca arundinacea* Schreb." *Journal of Experimental Botany* 25 (88):965-972.

Thorne, GN, and DW Wood. 1987. "Effects of radiation and temperature on tiller survival, grain number and grain yield in winter wheat." *Annals of Botany* 59 (4):413-426.

Tripathi, Satish C, KD Sayre, JN Kaul, and RS Narang. 2003. "Growth and morphology of spring wheat (Triticum aestivum L.) culms and their association with lodging: effects of genotypes, N levels and ethephon." *Field Crops Research* 84 (3):271-290.

Turgeon, Robert, and JA Webb. 1971. "Growth inhibition by mechanical stress." *Science* 174 (4012):961-962.

Uthayakumaran, S, and CW Wrigley. 2010. "Wheat: characteristics and quality requirements." In *Cereal Grains*, 59-111. Elsevier.

Venning, Frank D. 1949. "Stimulation by wind motion of collenchyma formation in celery petioles." *Botanical Gazette* 110 (3):511-514.

Verhertbruggen, Yves, Susan Marcus, Jianshe Chen, and Paul Knox. 2013. "Cell wall pectic arabinans influence the mechanical properties of *Arabadopsis thaliana* inflourescence and their response to mechanical stress." *Plant Cell Physiology* 0:1-11.

Wadsworth, R. 1959. "An Optimum Wind Speed for Plant Growth." *Annals of Botany* 23 (89):195-199.

Wang, Jian, Jinmao Zhu, Qinqin Lin, Xiaojuan Li, Nianjun Teng, Zhensheng Li, Bin Li, Aimin Zhang, and Jinxing Lin. 2006. "Effects of stem structure and cell wall components on bending strength in wheat." *Chinese Science Bulletin* 51 (7):815-823.

Wang, Yan-Hong, Fei-Hai Yu, Ming Dong, Xi-Qiao Lin, Hong Jiang, and Wei-Ming He. 2010. "Growth and Biomass Allocation of *Lolium perenne* Seedlings in REsponse to Mechanical Stimulation and Water Availability." *Annales Botanici Fennici* 47:367-372.

Whitehead, FH, and R Luti. 1962. "Experimental studies of the effect of wind on plant growth and anatomy: I. Zea mays." *New Phytologist* 61 (1):56-58.

Williams, RF, and Rosemary A Metcalf. 1975. "Physical constraint and tiller growth in wheat." *Australian Journal of Botany* 23 (2):213-223.

Wollenweber, B., J. R. Porter, and J. Schellberg. 2003. "Lack of Interaction between Extreme High-Temperature Events at Vegetative and Reproductive Growth Stages in Wheat." *Journal of Agronomy and Crop Science* 189 (3):142-150. doi: 10.1046/j.1439-037X.2003.00025.x.

Wrigley, CW. 2010. "An introduction to the cereal grains: major providers for mankind's food needs." In *Cereal Grains*, 3-23. Elsevier.

Yang, Dongqing, Tie Cai, Yongli Luo, and Zhenlin Wang. 2019. "Optimizing plant density and nitrogen application to manipulate tiller growth and increase grain yield and nitrogen-use efficiency in winter wheat." *PeerJ* 7:e6484.

Zhao, Benliang, Lili Teng, Jia-en Zhang, Huimin Xiang, Meijuan Li, and Kaiming Liang. 2018. "Thigmotropic responses of Oryza sativa L. to external rubbing stimulation." *Archives of Biological Sciences* 70 (1):129-139.

Zhao, Daqiu, Chenxia Han, Jun Tao, Jing Wang, Zhaojun Hao, Qingping Geng, and Bei Du. 2012. "Effects of inflorescence stem structure and cell wall components on the mechanical strength of inflorescence stem in herbaceous peony." *International journal of molecular sciences* 13 (4):4993-5009.